Fall 10-31-1993

Engineering design of an anaerobic-aerobic system to treat chlorophenols

Chih-Ju Jou
New Jersey Institute of Technology

Order Number 9409120

Engineering design of an anaerobic-aerobic system to treat chlorophenols

Jou, Chih-Ju, Ph.D.

New Jersey Institute of Technology, 1993

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

ENGINEERING DESIGN OF AN ANAEROBIC-AEROBIC SYSTEM TO TREAT CHLOROPHENOLS

by
Chih-Ju Jou

A Dissertation
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

Department of Chemical Engineering,
Chemistry, and Environmental Science

October 1993

APPROVAL PAGE

Engineering Design of an
Anaerobic-Aerobic System to Treat Chlorophenols

Chih-Ju Jou

Dr. Piero M. Armenante, Thesis Advisor — Date
Associate Professor of Chemical Engineering, NJIT

Dr. Gordon Lewandowski, Thesis Co-Advisor — Date
Professor of Chemical Engineering, and Chairperson of the Department of Chemical Engineering, Chemistry and Environmental Science, NJIT

Dr. David Kafkewitz, Committee Member — Date
Associate Professor of Biological Sciences, Rutgers University

Dr. Basil C. Baltzis, Committee Member — Date
Associate Professor of Chemical Engineering, NJIT

Dr. Ching-Rong Huang, Committee Member — Date
Professor of Chemical Engineering, NJIT

ABSTRACT

Engineering Design of an Anaerobic-Aerobic System to Treat Chlorophenols

by

Chih-Ju Jou

The present work was aimed at determining the effect of the main operating parameters on the performance of an anaerobic-aerobic reactor system to degrade toxic chlorinated compounds. In previous work conducted in the Biological Treatment Lab at NJIT it was shown that this system is capable of achieving the complete degradation of chlorophenols. In that system, reductive dehalogenation took place in the first anaerobic reactor, which was followed by a second aerobic reactor in which the degradation products of the first reactor were mineralized. In the present work the role of a number of parameters that can have a significant impact on the performance of the anaerobic reactor, namely, pH, temperature, concentration of pollutants, and residence time were examined.

The medium pH appears to have a very significant impact on the ability of the anaerobic organisms to dechlorinate. Experiments were conducted using a novel, chloride-free, completely defined medium in which the buffering agent was one of several non-fermentable buffering agents (MOPS, TRICINE, BICINE, CHES). The results indicate that the dechlorination process occurs only if the pH is within the range 8.0-8.8. In addition, stoichiometric amounts of chloride ion were produced during the process. The dechlorination process was also studied at different temperatures. It was found that the data could be interpreted assuming an Arrhenius kind of dependence for the degradation reactions on temperature.

The overall dechlorination reaction was mathematically modeled assuming that the degradation process is constituted of a series of single dechlorination steps. Rate constants for each step were obtained in independent experiments. The resulting model was then used to predict the rate of degradation in both batch and continuous reactors. The prediction of the model matched closely the experimental results obtained in such systems, thus confirming the validity of the kinetic mechanisms postulated in the development of the model.

BIOGRAPHICAL SKETCH

Author: Chih-Ju Jou

Degree: Doctor of Philosophy in Chemical Engineering

Date: October 1993

Undergraduate and Graduate Education:

- Master of Science in Chemical Engineering,
  New Jersey Institute of Technology, Newark, NJ, 1989

- Bachelor of Science in Chemical Engineering,
  National Central University, Taiwan, 1984

Major: Chemical Engineering

Publication:

- Armentante, P. M., D. Kafkewitz, C. J. Jou, and G. Lewandowski, "*Effect of pH on the Anaerobic Dechlorination of Chlorophenols in a Defined Medium.*" *Appl. Microb. & Biotechnol.*, in press, 1993.

Manuscript under preparation:

- Armentante, P. M., D. Kafkewitz, C. J. Jou, and G. Lewandowski, "*Kinetic Modeling of a 2,4,6-TCP Anaerobic-Aerobic Process.*".

Oral Presentations:

- Jou, C. J., P. M. Armenante, G. Lewandowski, and D. Kafkewitz, "*Engineering Design of an Anaerobic-Aerobic System to Treat Halogenated Aromatic Compounds Using Immobilized Microorganisms.*" Mini Tech Conference, Newark, NJ, Apr. 1992.

- Jou, C. J., P. M. Armenante, G. Lewandowski, and D. Kafkewitz, "*Aerobic Biodegradation of 3-Chlorophenol in an Anaerobic-Aerobic System.*" Mini Tech Conference, Newark, NJ, Apr. 1991.

Poster Presentation:

- Armentante, P. M., D. Kafkewitz, G. Lewandowski, C. J. Jou, C. M. Kung, M Larkin, S. Allen, and T. Kearney, "*Anaerobic-Aerobic Treatment of Chlorinated Aromatics.*" presented at the AIChE 1992 Annual Meeting, Miami Beach, FL, Nov. 1-6, 1992.

- Jou, C. J., P. M. Armenante, G. Lewandowski, and D. Kafkewitz, "*Anaerobic Dechlorination of 2,4,6-Trichlorophenol Using a Defined Medium.*" presented at the Air and Waste Management Association, Mid-Atlantic States Section Conference, Atlantic City, NJ, Sep. 30, 1992.

This thesis is dedicated to
Dr. Piero M. Armenante and Dr. Gordon Lewandowski

ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to his supervisors, Professors Piero M. Armenante, Gordon Lewandowski and David Kafkewitz , for their guidance, friendship and moral support throughout this research.

Special thanks to Professors Ching-Rong Huang and Basil C. Baltzis for serving as members of the committee.

The author is grateful to the Hazardous Substance Management Research Center for providing funding for this project.

The author appreciates the timely help and supports from his family members, including: Wife (Yi-Ling Lin), Father (Yen-Shong Jou), Mother (Su-Pi Yin Jou) and young brother (Jerry Jou).

TABLE OF CONTENTS

Chapter	Page

Chapter | Page

LIST OF FIGURES

Figure		Page

Figure		Page

LIST OF TABLES

Table		Page

Table		Page

Chapter 1
INTRODUCTION

Chlorophenols constitute an important class of compounds widely used in many industrial applications and commonly found in the effluents of many industrial plants such as paper mills, as well as in the soil of many industrial facilities. Chlorinated phenols have been used as antiseptics since the late eighteenth century, and some of them, such as 2,4,6,-trichlorophenol (2,4,6-TCP), and pentachlorophenol (PCP), are used as fungicides and preservatives. Most chlorophenols are produced directly by industry. However, they can also be formed during the breakdown of chlorobenzenes and the chlorination of water containing phenols. The presence of these compounds in water is often detected as a result of their medicinal odor.

Because of their toxicity and recalcitrance to traditional aerobic biodegradation, chlorophenols have been the focus of a number of recent studies and investigations targeted at determining their degradability under anaerobic conditions (Fathepure and Vogel, 1991; Kiyohara et al., 1992; Mohn and Kennedy, 1992; Nicholson et al., 1992; Battersby and Wilson, 1989; DeWeerd and Suflita, 1990; Holliger, et al., 1992; Mohn and Kennedy, 1992a; Mohn and Tiedje, 1990; Boyd et al., 1983; Boyd and Sheldon, 1984; Dietrich and Winter, 1990; Haggblom, and Young. 1990; Mohn and Tiedje, 1992).

One apparent limitation of anaerobic dehalogenation is that its effectiveness decreases in inverse proportion to the number of halogen atoms present in the molecule. That is, monochlorophenols are more difficult to dehalogenate than penta-, tetra-, or tri-chlorophenols (Hendriksen et al., 1992; Mohn and Kennedy, 1992a; Mohn and Kennedy, 1992b; Madsen and Licht, 1992; Woods et al., 1989; Kohring et al., 1989; Shang and Wiegel, 1990).

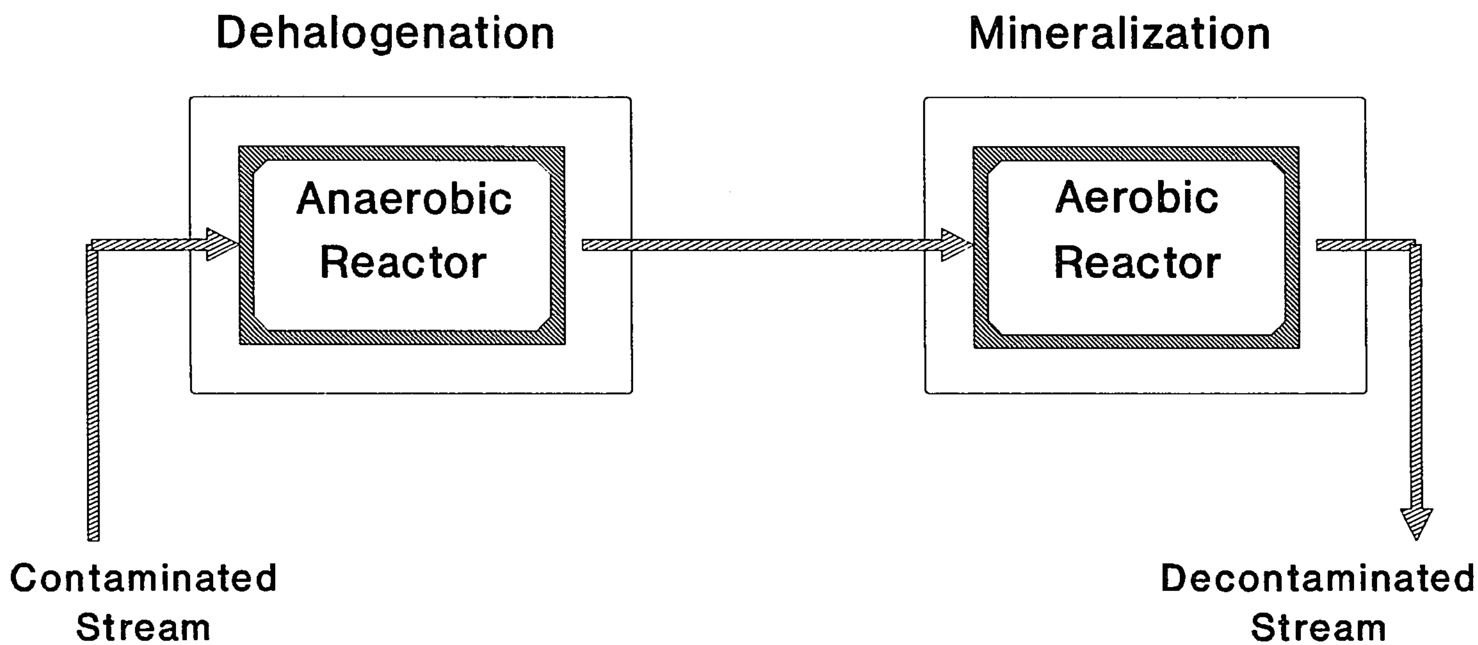

Figure 1-1 Schematic of an anaerobic-aerobic process

However, monochlorophenols can be successfully and rapidly degraded under aerobic conditions (Chaudhry and Chapalamadagu, 1991; Knackmuss, 1982). This has suggested the approach of the present work based on the development of a combined anaerobic-aerobic continuous process (Figure1-1) to produce the complete degradation of 2,4,6-TCP (Armenante, et al., 1992; Kafkewitz et al., 1992). In previous studies conducted in the Biological Treatment Lab at NJIT, it was shown that the 2,4,6-TCP contained in an aqueous stream could be sequentially and stoichiometrically converted to 2,4-dichlorophenol (2,4-DCP) and then to 4-chlorophenol (4-CP), by anaerobic dehalogenation. The 4-CP so generated could then be mineralized aerobically.

Armenante et al. (1992) have shown that anaerobic dechlorination can be significantly affected by a number of operating parameters. In that work, the feed to the anaerobic reactor was the clear, sterile supernatant from a liquor obtained from a local treatment facility, synthetically contaminated with 2,4,6-TCP. Although that work showed that the process is viable when real waste effluents are used, it did not define the essential medium requirements for anaerobic dehalogenation to occur. A review of the literature showed that, until recently (1991), undefined media have been used in most reports of anaerobic dehalogenation (Mohn and Kennedy, 1992a; Madsen and Aamand, 1992).

The work of Armenante et al. (1992) also indicated the sensitivity of the anaerobic-aerobic process to pH change. In particular, it was observed that the pH optima for the anaerobic and aerobic steps were different. The anaerobic dehalogenation reaction was accompanied by a sharp, and apparently necessary, rise in pH from 7.5 to 8.7. Any attempt to lower the pH to values at or below 6.8 using phosphate buffer additions (50 mM) severely inhibited dehalogenation. Higher phosphate concentrations completely inhibited dehalogenation. Whether the effect was due to pH effects or phosphate-induced inhibition was not established. Conversely, it was found that aerobic degradation of the 4-CP produced in the anaerobic step took place only if sufficient phosphate buffer was continuously added to the aerobic reactor to reduce and maintain the pH below 8.0. Hence, phosphate buffer addition had opposite effects on the anaerobic and aerobic populations.

Chapter 2
OBJECTIVES OF THIS WORK

The goal of this work was to answer some of the most important questions raised in Chapter 1. Therefore, the specific objectives of this work were to:

- develop a specific chloride-free defined medium for the anaerobic degradation of 2,4,6-TCP, chosen here as the model halogenated target compound. This step was required to close the mass balance during the anaerobic dehalogenation process since the amount of chloride ions released in the process could be detected;

- improve the overall degradation rate of 2,4,6-TCP by selecting the most effective microbial consortia to carry out the anaerobic dehalogenation and aerobic mineralization steps;

- optimize the operating conditions for each step of the process with specific attention paid to the effects of pH on both the anaerobic and aerobic processes and the effect of temperature on anaerobic dehalogenation;

- study different reactor configurations for the anaerobic dechlorination and aerobic mineralization steps;

- derive and test a mathematical model for each step in the process and explore its implications for scale-up of the combined process.

Chapter 3
LITERATURE REVIEW

3.1 Use of Defined Media in Previous Anaerobic Dehalogenation Studies

Dehalogenation using anaerobic bacteria has been reported in a number of publications. Although defined media have been used to conduct anaerobic fermentations (Long-de Vallere et al., 1989), most of the available references show that dehalogenation was observed in undefined media (for example, Tiedje et al., 1987, Edwards et al., 1990). A number of investigators have studied anaerobic dehalogenation in media that were completely defined except for the use of yeast extract (Taylor et al., 1979; DeWeerd et al. 1990 and 1991; Dolfing, 1990; Kamal and Wyndham, 1990; Dolfing and Tiedje, 1986). Very few papers reported dehalogenation in defined media until very recently (Holliger et al., 1992; Kennedy and Berg, 1982; Mandsen and Aamand, 1992; Mandsen and Licht, 1992).

Holliger et al. (1992) reported the successful anaerobic dechlorination of hexachlorobenzene (HCB), pentachlorobenzene (QCB), all three isomers of tetrachlorobenzene (TeCB), 1,2,3-trichlorobenzene (1,2,3-TCB), and 1,2,4-TCB in defined media. Mandsen and Aamand (1992) described the anaerobic transformation of TCPs (2,4,6-TCP, 2,3,5-TCP and 2,4,5-TCP) in a stable enrichment culture in a defined medium. Kennedy and Berg (1982) reported the anaerobic digestion of piggery waste using a stationary fixed film reactor fed with defined medium. Mandsen and Licht (1992) reported the isolation of an obligatory anaerobic bacterium that transforms several chlorophenols (CPs) in a defined medium.

3.2 Evidence of Anaerobic Dehalogenation

Horowitz et al. (1983) performed a series of experiments with halobenzoate compounds to demonstrate that dehalogenation reactions are brought about by anaerobes and not by abiotic photochemical reactions. They showed that reactions occur in the dark and under anaerobic conditions with live bacteria present. Sterilized sludge samples did not carry out any reaction even in the presence of titanium citrate, a strong reductant. Furthermore, disruption of the normal incubation temperature (>39^{o}C) inhibited the dehalogenation reactions in fresh water sediments. When halogenated aromatic compounds were added to unadpated sediments, lag periods with a low background of methanogenesis were followed by a phase of rapid degradation. Such observation indicated a specific biological adaptation, as seen with the acclimation of sediments by prior exposure to the halogenated aromatic compounds.

In general, the mineralization of halogenated aromatic compounds follows their complete dehalogenation. Therefore, it is important to understand the factors that influence the microbial attack of the halogenated compounds. There are similarities and differences between the aerobic and the anaerobic dehalogenation of aromatic compounds. For example, in both aerobic and anaerobic dehalogenation, the *meta*-substituted aromatic compounds tend to be most recalcitrant (Bollag et al., 1974). On the other hand, the positive correlation observed in aerobic systems between the persistence of the compounds against dehalogenation and the number of halogens (DiGeronimo et al., 1979; Okey and Borgan, 1965) is not apparent for anaerobic metabolism (Suflita et al., 1982).

Many studies have confirmed these two general observations. For example, the *Cl* substituents *ortho* to the phenolic group, according to Mikesell and Boyd (1986), are removed more rapidly than *Cl* in the *meta* and *para* positions. Their data show that it was more difficult to dehalogenate the lower chlorinated phenols, namely tetrachlorophenols (TeCPs) and trichlorophenols (TCPs), than pentachlorophenol (PCP). Dietrich and Winter's mixed cultures (1990) were able to dehalogenate 2-bromophenol, 2-chlorophenol (2-CP) and completely dechlorinate 2,6-dichlorophenol (2,6-DCP), whereas with 2,4-DCP, only the *ortho* substituents could be eliminated.

Hakulinen et al. (1985) examined the anaerobic degradation of 8 polychlorinated phenols (2,3-, 2,4-, 2,5-, 3,4-, 3,5-DCPs, 2,4,6- and 3,4,5-TCPs, 2,3,4,5-TeCP, and PCP). Their results ranged from no reaction of 3,4-DCP to 90-99% elimination of *ortho* chlorines from the TCPs and TeCPs.

The rate for each dehalogenation step differs among the halogens. Several studies (Suflita et al., 1982; Mikesell and Boyd, 1986; Boyd and Shelton, 1984; Boyd et al., 1983) showed that iodo- and bromo- substituents are degraded after a shorter lag time than their chloro- and fluoro- counterparts, which suggests that the iodo- and bromo- species are more readily dehalogenated.

Both the number and position of the halogen atoms play similar roles in the anaerobic degradation of polychlorinated biphenyls (PCBs) as in phenols. For example, the more highly substituted PCB metabolizes easier than the mono- and dichlorinated biphenyls (Larsson and Lemkemeier, 1989).

3.3 Anaerobic Techniques

There are several variations of Hungate's technique for cultivating anaerobic microorganisms (Bryant, 1972; Holdeman and Moore, 1972; Hungate, 1969; Macy et al., 1972). Serum bottles closed with a butyl rubber stopper and a crimped metal seal (Miller and Wolin, 1974) are used for cultivation. The media are prepared under sterile conditions, usually with an appropriate reducing agent. The media are typically purged with N_2 gas and added to the serum bottles. All inoculations are usually carried out with hypodermic syringes and needles.

3.4 Classification of Anaerobic Microorganisms

Anaerobic microorganisms may conveniently be divided into three general categories based on the organisms' trophic requirements (Barnes and Fitzgerald, 1987). The first comprise hydrolytic bacteria, commonly known as acidogens, because they initially ferment their substrate into short-chain organic acids and other small molecules. The second group, the heteroacetogens, produce acetic acid and hydrogen, and the third is that of the methanogens, which produce methane. The methanogens can be further divided into acetic acid users (acetotrophs) and hydrogen utilizers (lithotrophs). Furthermore, feed stocks containing sulfate sulfur and nitrate may lead to two additional groups of bacteria, the sulfate-reducers and the denitrifiers.

A number of anaerobic dehalogenations are methonogenic. The broad nutritional requirements for growth of methanogens (Schonheit and Thauer, 1979), namely carbon, nitrogen , phosphorus, sulfur, calcium, magnesium, potassium, sodium, trace organic nutrients (such as amino acids and vitamins), and trace metals (such as iron, zinc manganese, cobalt, molybdenum and nickel), have made methanogens an extremely fastidious group in the symbiosis of anaerobic digestion.

The majority of mesophilic methanogens do not grow at pH values below 5.5, because high acidity favors proton reduction of hydrogen rather than hydrogen reduction to methane, and therefore inhibits methane production. Empirical data have further shown that the upper pH limit for methanogenic activity to occur is 9.

3.5 Advantages of Anaerobic Degradation

Anaerobic processes offer several advantages over aerobic processes (Forday and Greenfield, 1983; Anderson et al., 1984, Long-de Vallere et al., 1989):

a. Anaerobic systems generate less sludge than aerobic systems. Aerobic processes typically yield about 0.5 to 1.5 kg of biomass (sludge) solids for each kg of biological oxygen demand (BOD) removed; while anaerobic processes yield about 0.1 to 0.2 kg per kg of BOD removed.

b. The aeration energy required by aerobic processes exceeds the mixing energy required for anaerobic processes, thus making anaerobic processes more energy efficient.

c. Methane, an anaerobic product, can be utilized as a source of fuel.

d. Anaerobic systems are better suited to dehalogenate aromatic compounds for the following reasons:

1. The decrease in the electron density of the aromatic nucleus enhances anaerobic enzymatic attack by a reductive (nucleophilic) mechanism (Klinman, 1972);

2. Halogenated aromatic compounds tend to polymerize when oxidized by aerobic bacteria. The polymers are rather resistant to further

bacteria attack (Knackmuss, 1982). Anaerobic processes, on the other hand, prevent oxidation and polymerization, hence enhance degradation.

In addition, anaerobic microorganisms are capable of being dormant for a long period (2-3 months), and then being fully operational within 2-3 days (Armenante et al., 1992 and Kafkewitz et al., 1992).

Several socio-economical factors have lead to more support for anaerobic treatment, namely: the need to reduce energy costs of treatment, particularly when biologically treating high strength waste waters (since no aeration is required), the more stringent requirements to pretreat industrial waste waters prior to sewer discharge, and the unsuitability of alternative treatment methods for some types of waste waters.

3.6 Determination of the Products of Anaerobic Biodegradation

Anaerobic transformation sometimes produces intermediates and end-products that are more hazardous than the parent compounds (Edwards et al., 1990). An example is vinyl chloride produced from the dehalogenation of chloroform. Therefore, it is crucial to monitor the degradation products. Several techniques have been in use to monitor the formation of hazardous intermediates during the degradation process. For example, the concentration of the transformed compounds in the effluent can be directly determined by GC or HPLC. The chloride ion formation can be determined by using the chloride-ion electrode (Radehaus and Schmidt, 1992). The degradation products can be determined by GC-MS, if complete mineralization has not occurred. Measurements of gas production are also an indirect method to assess the degree of degradation via mass conversion of organic carbon to carbon dioxide and methane (Long-de Vallere et al., 1989; Shelton and Tiedje, 1984a). For example, Hakulinen and

Salkinija-Salonen (1982) measured the amount of $^{14}CO_2$ to ascertain the complete mineralization of ^{14}C PCP.

3.7 Isolation of Dehalogenating Pure Cultures

Several groups have isolated pure bacterial cultures capable of metabolizing PCP under aerobic conditions. Among them are several strains of *Coryneform* (Chu and Kirsch, 1972), *Flavobacterium* (Saber and Crawford, 1985), and *Pseudomonas* (Wantanabe, 1973). However, no single anaerobic bacterium with the ability to degrade PCP has been isolated or identified (Mikesell and Boyd, 1986). Very recently, Madsen and Licht (1992) has isolated an obligatory anaerobic bacterium capable of degrading some of chlorobenzenes.

The difficulty in isolating a pure culture is attributed to the complexity of the microbial attack process against the toxic compounds. A single anaerobic microorganism is rarely able to mineralize a complex organic molecule. Rather, a succession of specialized organisms modifies the molecule in turn, each deriving a small amount of carbon and/or energy from the reaction (Hamilton, 1979; Lovely and Klug, 1982; Sleat and Robinson, 1984).

There have been many attempts to isolate single anaerobic cultures. For example, Dietrich and Winter's (1990) effort to isolate an anaerobic 2-CP dechlorinating organism failed. Instead a mixed culture was identified with three morphologically distinctive microorganisms. When Hakulinen et al. (1985) mixed the isolated cultures of *Pseudomonas aeruginosa* and *Klebsiella oxytoca*, the mixture was able to utilize 2,4,6-TCP as a sole carbon and energy source under anaerobic conditions. However, individually, none of the isolates were able to degrade 2,4,6-TCP under anaerobic conditions. Shelton and Tiedje's three pure cultures (1984b), which perform different reaction steps in the mineralization pathway of 3-chlorobenzoate, grow on 3-chlorobenzoate only upon mixing.

These studies suggest that anaerobic mixed microbial cultures provide the best hope for efficient and robust treatment.

3.8 Enrichment Sources

The first and sometimes the most time consuming step in developing a microbial degradation system is the search of the appropriate enrichment for selected microorganisms. It is important therefore to initiate sampling from the relevant sources in order to achieve maximum efficiency. The major sources, according to Berry's review (1987), include: soil, marine mud, sewage, subsurface, sludge, marine or lake sediments.

Soil and water samples chronically polluted with the compounds of interest are a major source of inocula to isolate organisms capable of attacking those compounds (Muller and Lingens, 1986). Organisms that are capable of either aerobic or anaerobic dechlorination are found in sewer systems. The extent of dechlorination also varies in each case study. For example, some PCBs (Tiedje et al., 1987), and hexachlorobenzes (Fathepure et al., 1988) are dechlorinated by river sediments and sewage sludge, respectively. The complete conversion of halobenzoic acids and halophenolic compounds to methane by lake sediment and sewage sludge microorganisms has been observed (Sahm et al., 1986). Bacterial strains from sediments of the PCB-contaminated New York Hudson river are able to degrade the majority of the congeners included in a commercial polychlorinated biphenyl oil (Bedard et al., 1986).

Chlorinated phenols and guaiacols are able to be degraded by mixed bacterial cultures obtained from areas polluted by bleach plant effluents containing these compounds. Complimentarity seems also to be a characteristic of these microorganisms, as mixed bacterial cultures originating from soil contaminated by tetrachlorinated phenols also degraded PCP (Valo et al., 1985).

Horowitz et al. (1983) found that before the anaerobic degradation of halobenzoate occurred, the lag time in an unacclimated sediment ranged from 0.4 to 40 weeks. They concluded that the lag period varies according to the type and position of the aryl halide. The substrate concentration also has an effect on the length of the lag period for 4-amino-3,5-dichlorobenzoate. In a subsequent study, Edwards et al. (1990) observed that a long lag period is often associated with the onset of anaerobic transformation, which is likely due to the presence of microorganisms in low numbers in aquifer solids and ground water. Confirming Horowitz's result of 1983, the concentration of the toxic compounds also affect the lag period. Furthermore, anaerobically digested municipal sewage sludge which has been acclimated to CP degradation for more than 2 years has been demonstrated to degrade PCP (Mikesell and Boyd, 1986).

3.9 Cometabolism

The transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound is known as cometabolism. This phenomenon is important in both the aerobic and anaerobic biodegradation of pesticides.

In many instances it appears that several distinct organisms are responsible for substrate degradation, each performing specific modifications on the substrate. In such instances, no single culture is able to use the substrates as sole carbon and energy source; therefore, it is necessary to add an alternative carbon source to support growth.

In aerobic systems, the transformation of trichloroethylene (TCE) is a cometabolic process (Edwards et al., 1990). TCE is not utilized by the bacteria, but only fortuitously transformed via methane monooxygenase reaction.

When Golovleva and Skryabin (1981) utilized a *Pseudomonas* to degrade DDT, the DDT did not serve as a source of carbon and/or energy. Hence alternative sources of carbon and energy, namely lactic acid and nitrate, were added to the medium.

In Slonim's work (1985) to degrade 4,6-dinitro-o-cresol (DNOC) in a continuous system, the performance of the anaerobic system was highly dependent on the influent concentration of the co-substrate sucrose. An influent stream having a sucrose-to-DNOC concentration ratio of 2:1 or higher resulted in a 95-100% removal (or conversion) of DNOC under anaerobic conditions. However, when the influent sucrose-to-DNOC ratio was less than 2:1, the anaerobic microorganisms failed to cometabolize DNOC.

3.10 Dehalogenation Pathways

Recently, several CPs have been shown to be anaerobically biodegradable. Nevertheless, neither the biochemical pathways nor the organism(s) responsible for degradation have been unequivocally identified (Hakulinen et al., 1985). The proposed degradation pathways under strict anaerobic conditions involve reductive dehalogenation (Suflita et al., 1982), and the addition of a hydroxyl group to the aromatic ring (Taylor et al., 1970). Suflita et al. (1982) demonstrated that the primary degradative event in the dehalogenation of benzoate was the deletion of the aryl halide with retention of the aromatic ring. Dehalogenation required strict anaerobic conditions and depended on the halogen and its position. The evidence suggested that the reductive nature of aromatic dehalogenation could be of some significance in the removal of chlorinated xenobiotics from the environment.

Aerobic metabolism of aromatic compounds, on the other hand, is characterized by:

a) occasional non enzymatic loss during NIH shifts (Guroff et al., 1967);

b) removal of the halogen from the alkyl moiety after cleavage of the ring (Goldman et al., 1967; Hovath and Alexander, 1970; Spokes and Walker, 1974; Hartmann et al., 1979; Reineke and Knackmuss, 1980); and

c) direct substitution of the halogen by a hydroxyl group (Johnson et al., 1972; Klages and Ligens, 1979. In the aerobic degradation of PCP, evidence (Suzuki, 1977; Rott et al., 1979) shows that the process proceeds by substitution of a chlorine substituent by a hydroxyl group, although the mechanism remains unclear.

The anaerobic and aerobic degradation of PCP by heterogeneous microbial cultures have been reported in many studies. Murthy et al. (1979) found that CO_2 formation was severely reduced under anaerobic conditions, with 2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,6-TCP, and pentachloroanisole as degradation products. Ide et al. (1983) and Boyd et al. (1983) identified 2,3,4,6-TeCP, 2,4,5-TCP, 3,4- and 3,5-DCP, and 3-CP as PCP degradation products. These results firmly indicated that sequential reductive dechlorination could be performed by microorganisms degrading PCP under anaerobic conditions.

3.11 Evidence of Aerobic Microorganisms in Anaerobic Systems

There are microorganisms, such as *Pseudomonas aeruginosa*, that exist in anaerobic reactors and are classified as aerobes (Hakulinen et al., 1985). Other investigators (McCarthy et al., 1962; Toerien et al., 1967; Taylor et al., 1970; Ferry and Wolfe, 1976) have also isolated similar species under anaerobic conditions. Taylor, as well as Ferry and Wolfe have isolated such aerobes from anaerobic cultures fed on aromatic compounds.

When Hakulinen's mixed cultures were tested with 2,4,6-TCP under anaerobic conditions, none of the isolates were able to degrade 2,4,6-TCP nor use it as a source of carbon or energy in pure culture. It seems that the presence of *Klebsiella oxytoca* enables the *Pseudomonas* strain to survive under anaerobic condition, and the two organisms develop a symbiotic relationship whose goal is to obtain a source of carbon.

3.12 Types of Immobilized Packed-Bed Bioreactors

An immobilized packed-bed bioreactor consists of a biofilm which grows on the solid supporting media. The purpose of the supporting media is to retain the biomass and protect the microorganisms against concentrated toxic compounds or washouts. Although there are restrictions (Mosey, 1977) on this type of reactor, researchers (Anderson et al., 1984), attracted by the numerous advantages of anaerobic systems, have used anaerobic packed-bed reactors to treat industrial waste waters.

(a). Upflow Pack- Bed Reactor

This reactor generally creates a plug flow pattern. However, the rising bubbles caused by gas production tend to stir up the flow pattern. The reactor is generally operated without recycle, but periodic backwashing may be required to remove accumulated solids (Barnes and Fitzgerald, 1987).

Krumme and Boyd (1988) used the upflow configuration with efficiencies greater than 90% to degrade a mixture of three CPs (2-CP, 3-CP and 4-CP) and 3,4,5-TCP. However, there was little biodegradation of either 2,4,6-TCP or PCP at the same operating conditions. The substrate loading rate was 20 ppm per day, at a hydraulic retention time of 2 to 4 days.

(b). Downflow Packed-Bed Reactor

The problem of clogging, as seen in upflow filters, may be overcome by using solid support materials of high void volume and irrigating the waste water over the media. These reactors can be operated with the liquid partially or completely filling the reactor. Previous studies suggest that biogas production plays a significant role in reactor mixing by increasing the axial dispersion (Kennedy and Berg, 1982; and Berg, 1984). However, recent data indicate that the major contribution to reactor mixing, instead of biogas production, comes from recycling (Long-de Vallere et al., 1989).

3.13 Anaerobic-Aerobic Biological Treatment Process

A promising alternative to wastewater treatment is the integrated anaerobic-aerobic system. One of the advantages of the anaerobic-aerobic integrated system is that it can treat compounds which are recalcitrant to conventional aerobic treatment, by aerobically treating the metabolic products of the first anaerobic process (Hakulinen and Salkinija-Salonen, 1982; Su, 1990; and Armenante et al., 1992). Hakulinen and Salkinoja-Salonen (1981) demonstrated that an anaerobic-aerobic fluidized bed process is able to reduce the concentration of organics and chlorinated phenolics in a pulp mill bleaching effluent. It was observed that the CPs are degraded in the first anaerobic stage, followed by toxicity and BOD removal in the aerobic filter. Ying et al. (1990), also developed a two-stage anaerobic-aerobic biological activated carbon (BAC) process for treating high concentration wastewaters from phenolic resin manufacturing. During the first anaerobic stage of decontamination; more than 90% of chemical oxygen demand (COD, from 30,000 mg/l), and more than 99% of the major constituents - phenol (12,000 mg/l), formaldehyde (3,000 mg/l) and methanol (2,500 mg/l) were removed. The second aerobic stage removed virtually all the remaining phenol and more than 90% of the residual COD (organic acids resulted from anaerobic degradation). Su (1990) also used an anaerobic-aerobic continuous stirred tank reactor (CSTR) for treating waste water containing high concentrations of nitrite and nitro-aromatic compounds resistant to aerobic degradation. The nitrite and nitro-aromatic compounds are successfully biotransformed to nitrogen and amino derivatives, respectively, in an anaerobic reactor. The amino derivatives were then degraded in the aerobic reactor. Armenante et al. (1992) also showed that an anaerobic-aerobic continuous process which included an anaerobic immobilized packed-bed reactor and an aerobic cell-suspended reactor was able to mineralize 25 ppm of 2,4,6-TCP in about 4 days.

Another advantage of the anaerobic-aerobic integrated system is that it can degrade volatile compounds that are susceptible of being stripped by aeration (Dienemann et al., 1990). Aerobic treatment alone may be unacceptable because volatilization of priority pollutants would pose a significant secondary air pollution threat. Dienemann, therefore, used serial anaerobic-aerobic packed-bed bioreactors to degrade organic contaminants in leachate from a high priority superfund site in the US. The reactor was configured to biodegrade the majority of the volatile species anaerobically, minimizing subsequent volatilization losses in the aerobic column. Slonim et al. (1985) used an anaerobic recycle fluidized bed reactor as a pretreatment stage for DNOC treatment, followed by an activated sludge reactor as the aerobic stage to mineralize the compound.

Chapter 4
CHEMICALS, MATERIALS, EQUIPMENT AND METHODS

4.1 Experimental Chemicals, Materials and Equipment

4.1.1 Experimental Chemicals

The chemicals used in the experimental part of this work are listed in Table 4-1.

Table 4-1 Chemical compounds used for experiments

Chemical Species	Supplier	Cat. Number	Note
2,4,6-TCP	Sigma	T 1266	
2,4-DCP	Sigma	D 6023	
4-CP	Sigma	C 4914	
MOPS	Sigma	M 9027	
TRICINE	Sigma	T 0377	
BICINE	Sigma	B 3876	
CHES	Sigma	C 2885	
Potassium Phosphate	Fisher	914778	
Potassium Phosphate Monobasic	Fisher	922644	
Ammonium Sulfate	Fisher	914822	
Magnesium Sulfate	Fisher	912214A	
Ferrous Sulfate	Fisher	792266	
Sodium Bicarbonate	Baker	31047	
Sodium Acetate	Baker	38412	
Sodium Formate	MC/B	SX570 CB723	
Resazurin	Kodak	P2106	
Nitrogen Gas	Liquid Carbonic	7727-37-9	Zero Grade Gas
Sodium Hydroxide	Aldrich	36717-6	

4.1.2 Experimental Materials and Equipment

Table 4-2 provides a list of the materials used in this work.

Table 4-2 Materials used for experiments

Equipment	Supplier	Cat. Number	Note
Serum Bottles	Fisher	06-406K	Size: 125 ml
Aluminum Seal Stoppers	Bellco	2048-11800	
Aluminum Seals	Bellco	2048-11020	Diameter: 20 mm
Seal Crimper	Bellco	2048-10020	
Silicone Rubber Tubing	Manosil	P8497-42	i.d.: 3/16" o.d.: 1/16"
Silicone Beads	Manville	R-635	Immobilization Support
Peristaltic Pump	Microperpex LKB	2132	Anaerobic-Aerobic System
Pump Driver	Cole Parmer	7543-12	Anaerobic Recirculation System

4.2 Experimental Equipment and Methods

4.2.1 Anaerobic and Aerobic Reactor Systems

4.2.1.1 Anaerobic Reactor Systems

4.2.1.1.1 Batch Culture Systems

Batch studies (Figure 4-1) were initial carried out in serum bottles to study the dehalogenation of 2,4,6-TCP. This work was performed in 125 ml serum bottles which were used as batch reactors, after being sealed with butyl rubber stoppers and aluminum crimp seals. Sampling was carried out by removing 3 ml through a syringe inserted through the butyl rubber stopper.

4.2.1.1.2 Immobilized Batch Reactor

The immobilized batch reactor (Figure 4-2) was a lucite cylinder, 5" in diameter and 18" in height, filled with R-635 silica beads (Manville Celite catalyst carrier) as a microbial support. The top and the bottom of the reactor were sealed with lucite plates using a silicon rubber sealant. The top side had a hole in the center, provided with a butyl rubber stopper. There were 4 sampling ports, 4" apart along the side of the reactor. The highest one was 4" below the top, and the lowest one was 2" above the bottom. The substrate was added from the top of the reactor. With a void fraction of 0.4 (Pak, 1988), the true operating volume was 2,000 ml (2.0 liters). To release the gases produced by the anaerobic microorganisms, an opening was made in the top of the reactor. Tubing was connected to this opening, with the other end immersed in water in order to provide a seal.

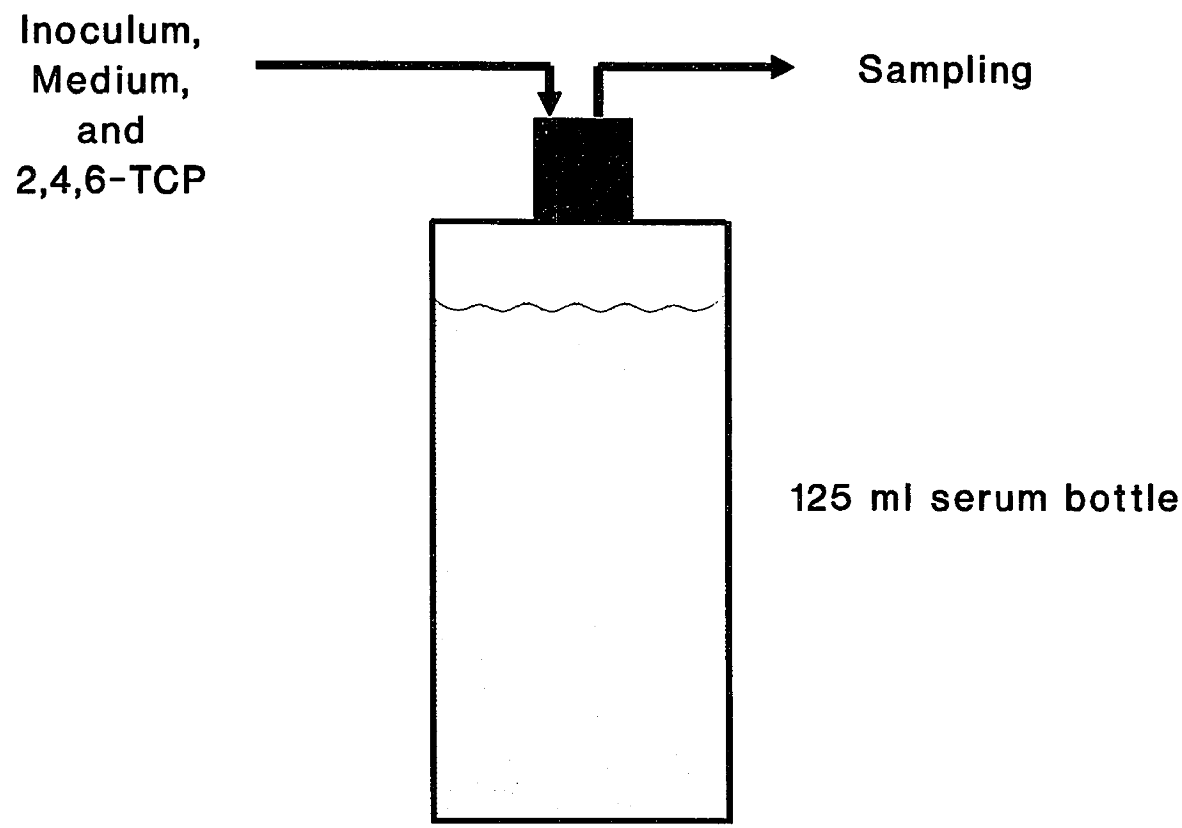

Figure 4-1 Schematic of an anaerobic batch process

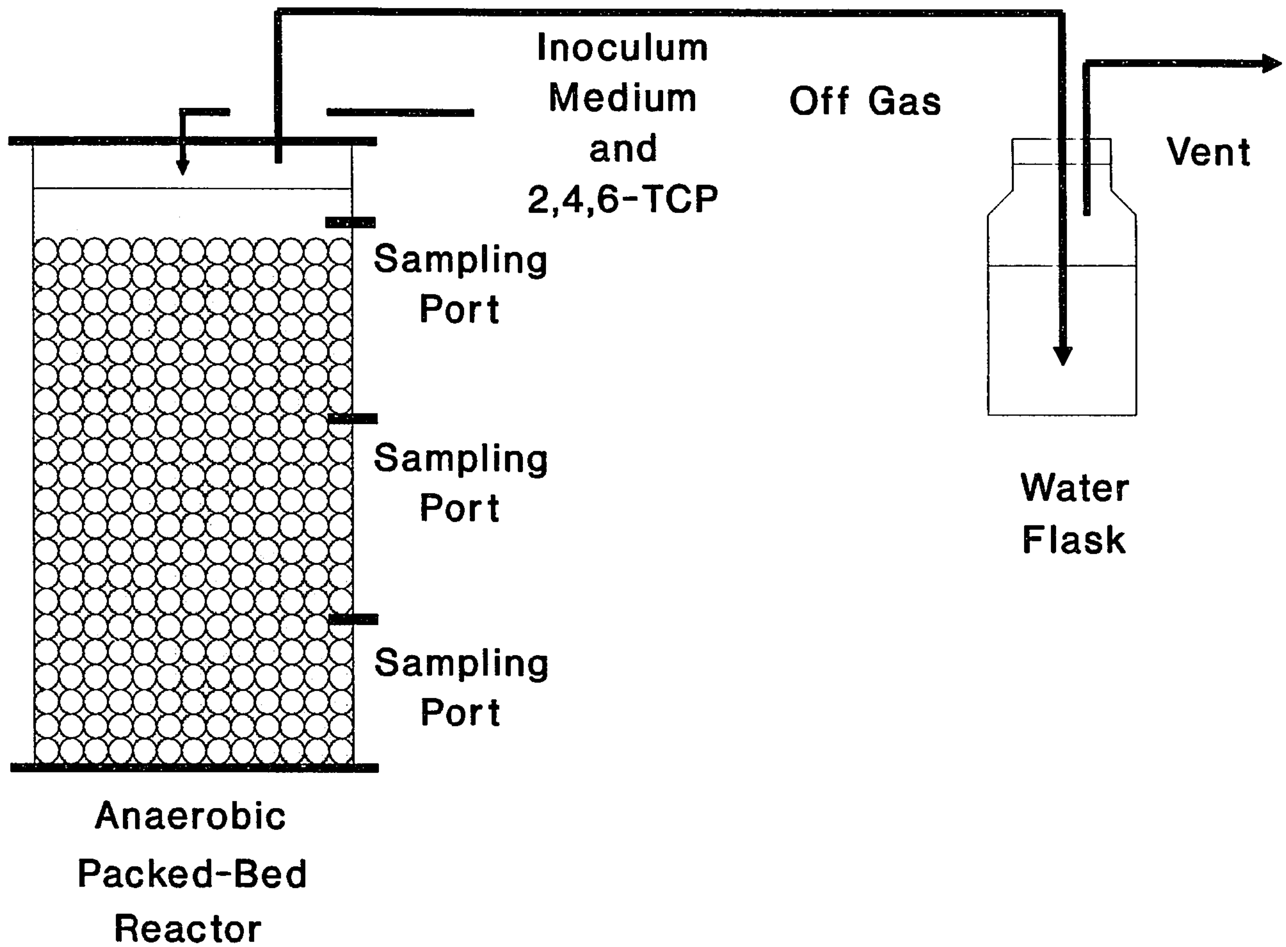

Figure 4-2 Schematic of an anaerobic immobilized batch react

4.2.1.1.3 Immobilized Recirculating Batch Reactor

The immobilized recirculating batch reactor (Figure 4-3) was operated by continuously recirculating the liquid content of the reactor using a Cole-Parmer pump. The pump drew liquid from the lowest sampling port of the reactor and transferred it to the top of the reactor, thus ensuring good internal mixing of the reactor liquid content. The recirculation rate was 25 ml/min.

4.2.1.1.4 Immobilized Recirculating Continuous Reactor

This immobilized recirculating continuous reactor (Figure 4-4) was similar to the immobilized recirculating batch reactor with the addition of a two-channel LKB peristaltic feed pump to continuously add and remove material. The flow direction of the continuous system was downward. The I/O ports were located at the center of the top cover of the reactor and 2" apart from the bottom. Samples could be taken from the three higher sampling ports, but typically they were taken from the output port (the bottom I/O port). The immobilization beads completely filled the reactor to within approximate 2.2" of the top. The input port (the top I/O port) of the reactor has a glass tube passing through the butyl rubber stopper. At the output port, there was a rubber tube, and a T-connector, one leg of which was the recirculating line while the other went either to a sample collector or the aerobic reactor. Throughout the process, the input and output flow rates were controlled by a two-channel LKB peristaltic pump so that they were equal.

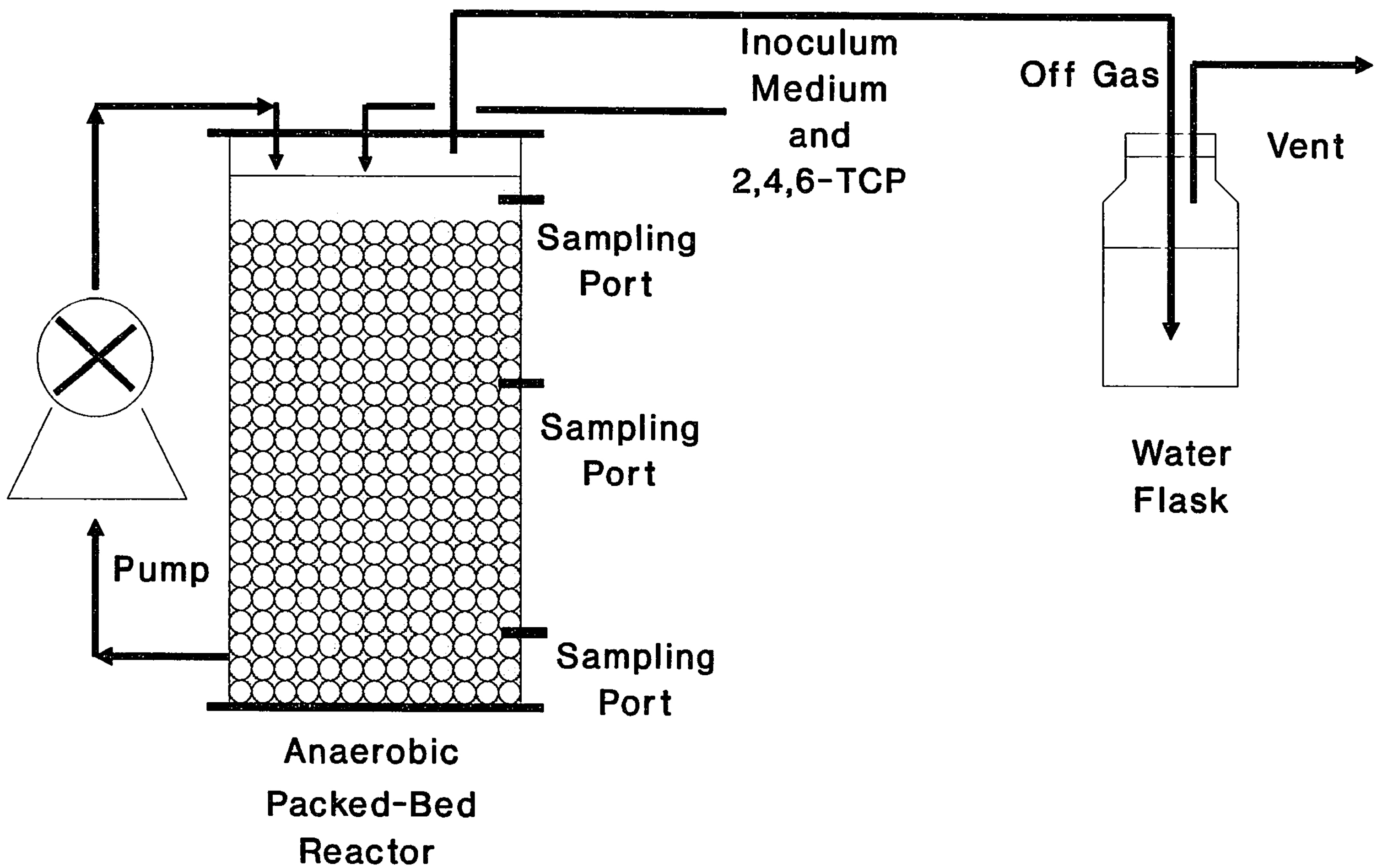

Figure 4-3 Schematic of an anaerobic immobilized recirculating batch reactor

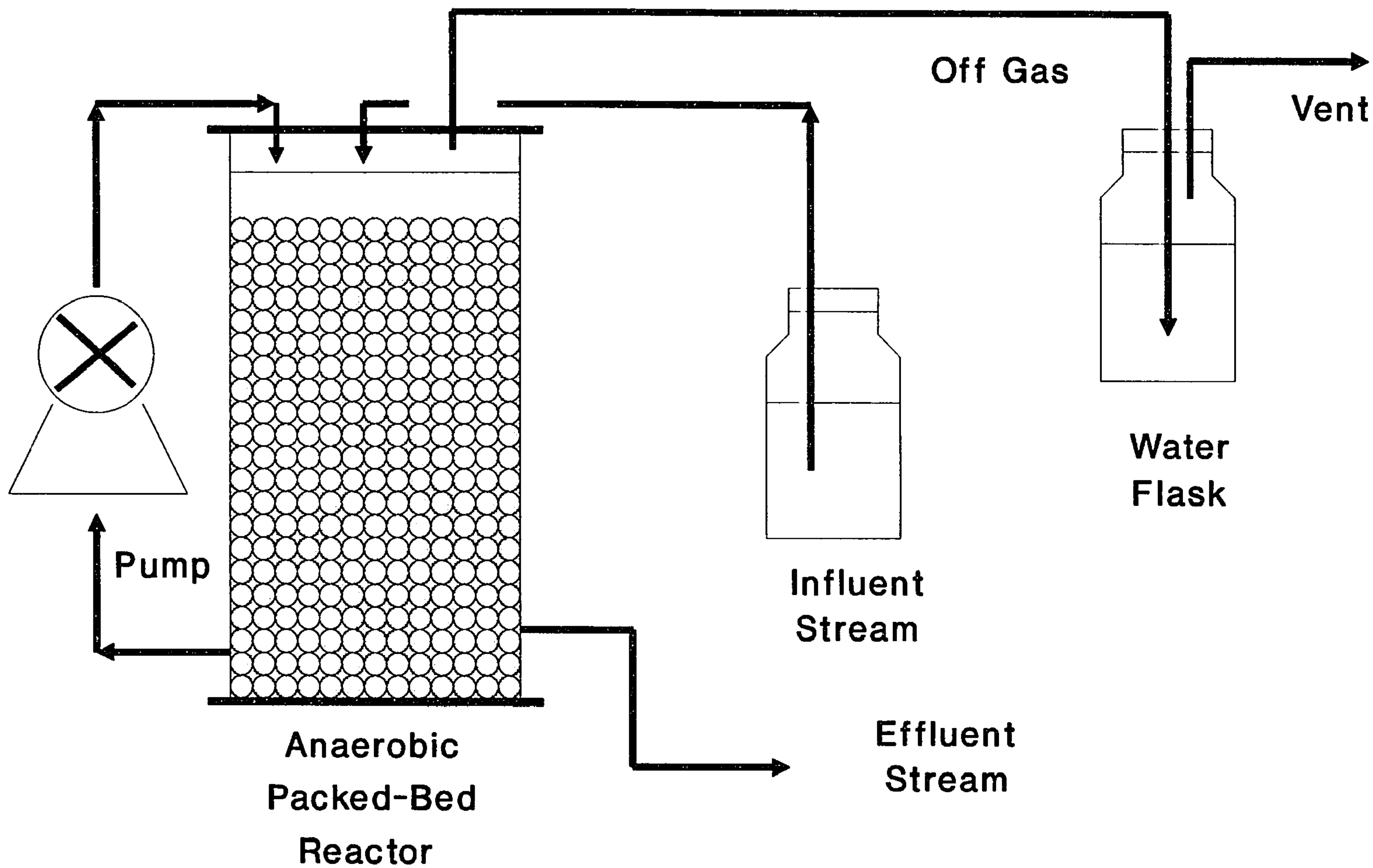

Figure 4-4 Schematic of an anaerobic immobilized recirculating continuous reactor

4.2.1.2 Aerobic Reactor Systems

4.2.1.2.1 Batch Culture Systems

200 ml shake flasks covered with loose plastic caps so as to allow oxygen from the environment to enter were used as batch reactors (Figure 4-5). The flasks were placed in a New Brunswick Controlled Environment Incubator Shake at 30 ^{o}C in the dark. The flasks were buffered with about 250 mM of MOPS to maintain the pH in the range 7.0 to 7.5.

4.2.1.2.2 Suspended Cell Continuous Reactor

The suspended cell continuous reactor (Figure 4-6) was a glass cylinder, 2" in diameter and 15" in height. There was no packing, and the aerobic microorganisms were suspended by bubbling air at an aeration rate of 50 ml/min. The operating volume of this aerobic reactor was about 770 ml. Both ends of the reactor were sealed with rubber stoppers. There were two inlets and one outlet for this aerobic reactor. One inlet was used to feed the substrate and was located in the middle of the reactor, while the other was used for aeration purposes and was located near the bottom of the reactor. The only outlet for collecting samples was located at the top of the reactor. The flow direction of the system was upward. The inlet used for substrate feed was provided with a T-connector connected to two sources: the effluent from the anaerobic reactor, and a 2.5 M MOPS buffer solution. The flow rate of these two inlet sources were in a ratio of 10:1, so that the final concentration of the biological buffer in the aerobic reactor was about 250 mM. The MOPS solutions was also delivered by a LKB peristaltic pump.

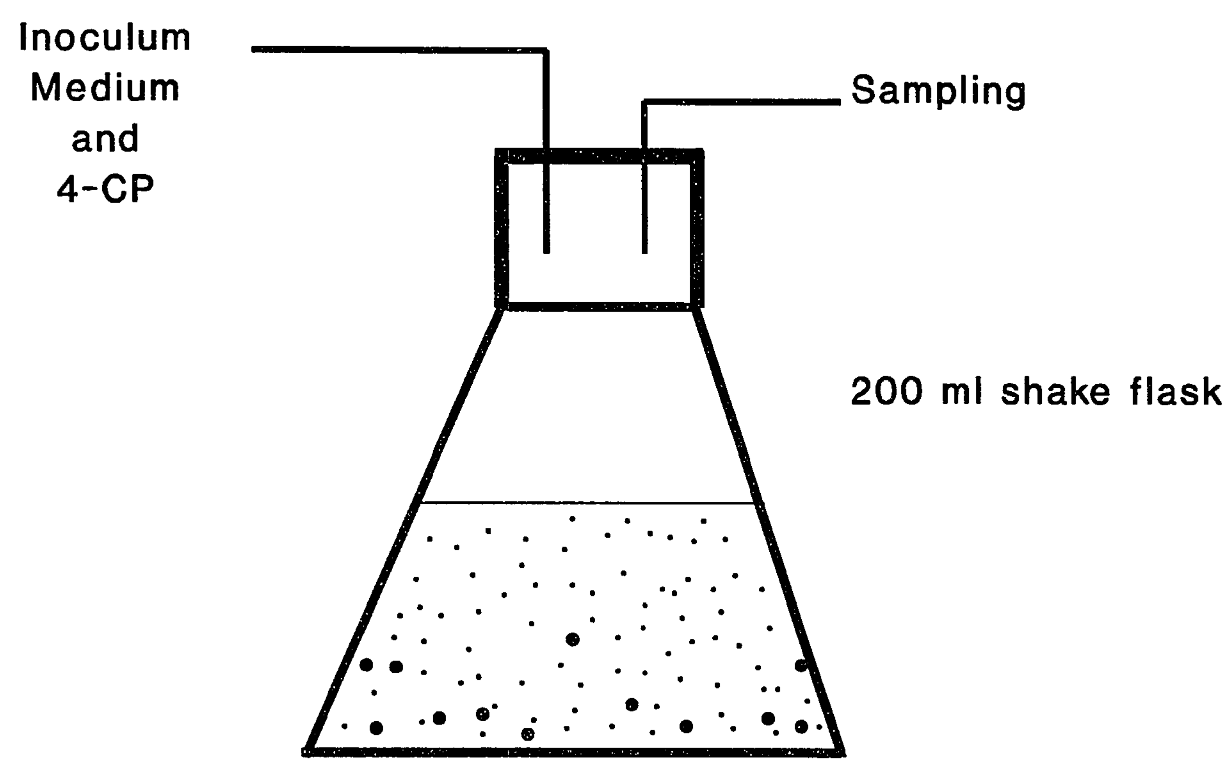

Figure 4-5 Schematic of an aerobic batch process

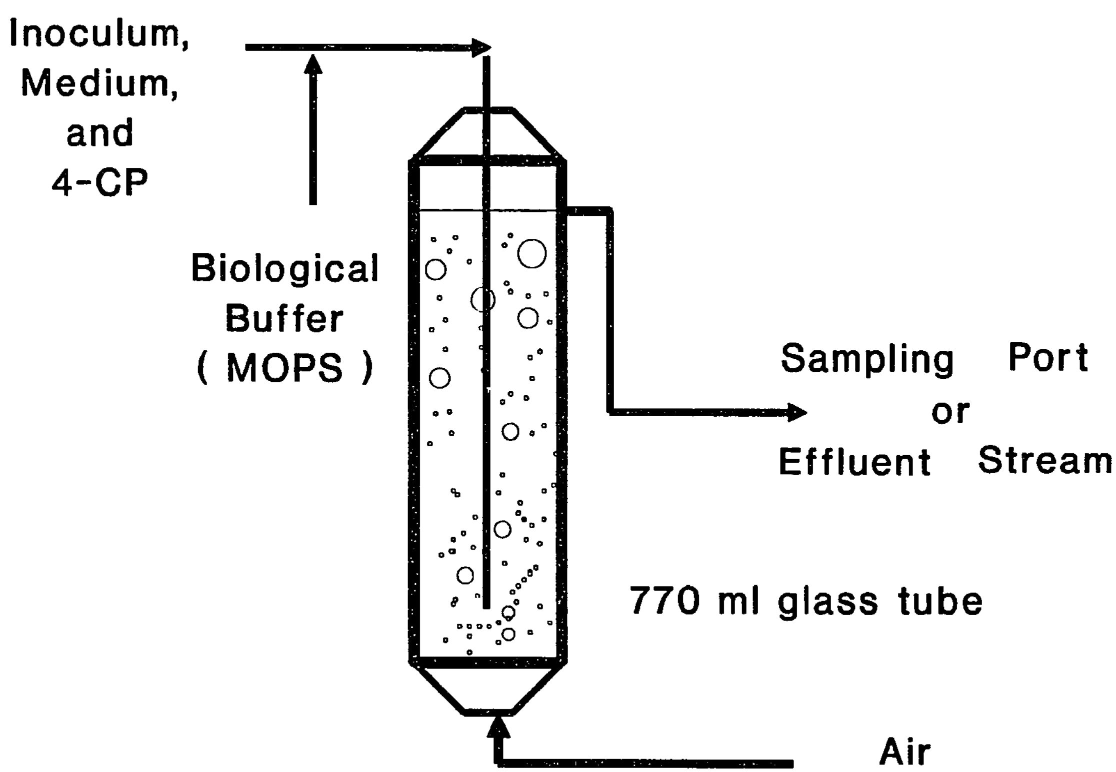

Figure 4-6 Schematic of an aerobic suspended cell continuous reactor

4.2.1.3 Anaerobic-Aerobic Continuous Reactor System

This anaerobic-aerobic continuous reactor system (Figure 4-7) was a combination of the anaerobic immobilized recirculating continuous reactor and the aerobic suspended cell continuous reactor described in the previous sections, in which the stream leaving the anaerobic reactor constituted the feed for aerobic reactor.

4.2.2 Preparation of Defined Media and Inoculum

4.2.2.1 Preparation of Defined Media

The composition of the defined media used for the dehalogenation of 2,4,6-TCP are shown in the Table 6-1. The stock solution of 2,4,6-TCP was 2,500 ppm in 0.1 NaOH. A 0.1 % stock solution of resazurin was also prepared in de ionized water.

The desired amount of 2,4,6-TCP and 0.0001% resazurin were added to the defined medium, and then boiled for 15 minutes to pre-reduce the medium. The medium was then placed in a ice batch while being purged with N_2 gas until cooled down to room temperature (measured with a mercury thermometer). It was found that there was no 2,4,6-TCP loss during the boiling and cooling processes. The boiled defined medium was transferred to 125 ml serum bottles or 2,000 ml flasks continuously flushed with N_2 gas. The serum bottles were sealed with butyl rubber stoppers and aluminum crimps, then autoclaved for 20 minutes at 121 ^oC and 15 psig. After autoclaving, the medium still has a pink color indicating the presence of oxygen. Therefore, the anaerobic defined medium was purged again with N_2 gas by repeated injection of fresh nitrogen gas and removal of the gas above the liquid in the bottle. The same procedures

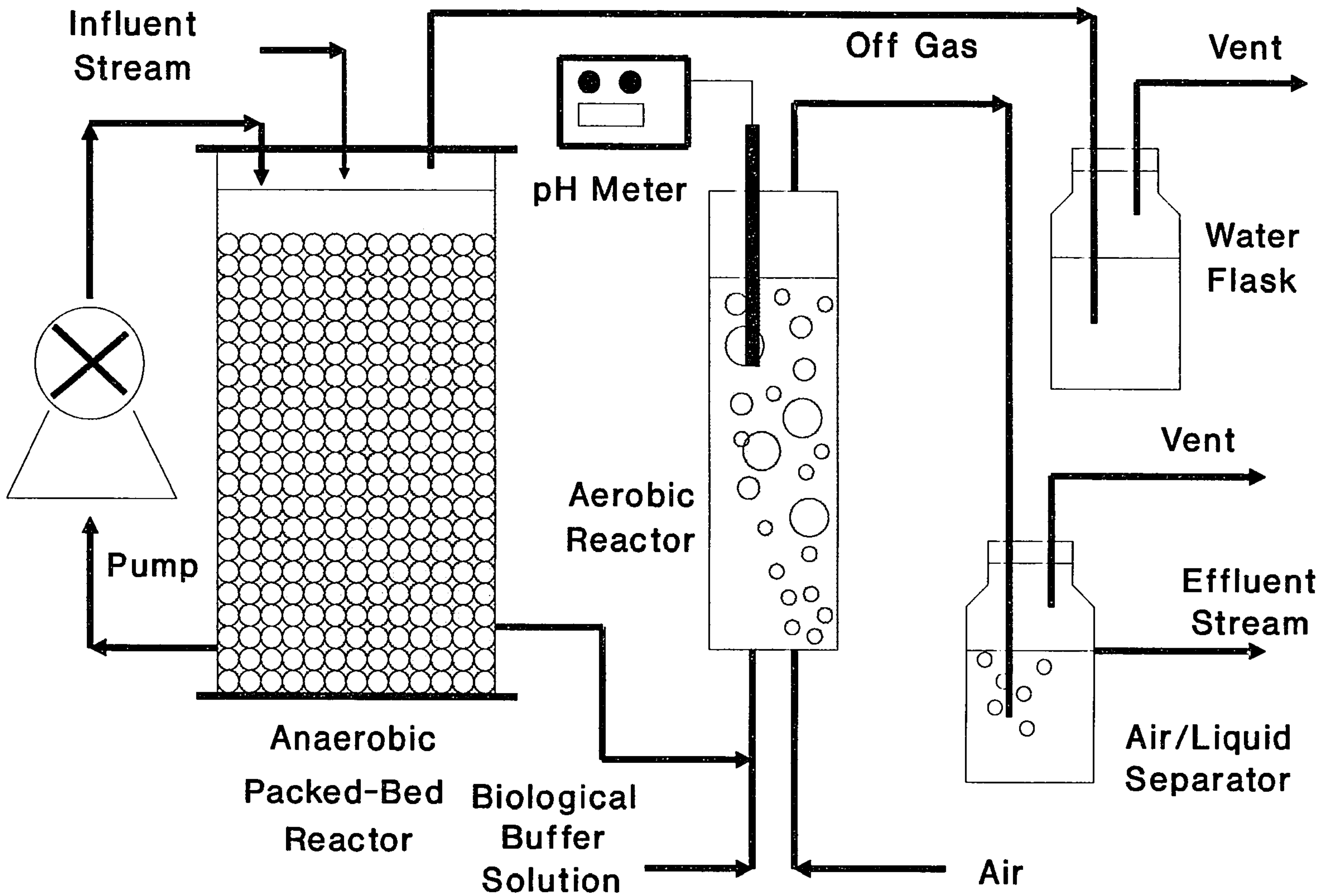

Figure 4-7 Schematic of an anaerobic-aerobic continuous reactor

were applied for the preparation in flasks except for a butyl rubber stopper that was used to seal the flask without using the aluminum crimp.

4.2.2.2 Inoculum

The first anaerobic enrichment culture for dehalogenation of 2,4,6-TCP in defined media was obtained from Kung's reactor series number R3 (Kung, 1991; and Armenante et al. 1992) in the Biodegradation Laboratory of New Jersey Institute of Technology. Originally the culture came from the anaerobic sludge obtained from the Joint-Meeting Treatment Plant in Elizabeth, New Jersey.

Two series of subcultures were estabilished by inoculation from Kung's bioreactor. One series was maintained in Medium A (as described below) by weekly or semiweekly sequential transfer. Second series of subcultures was similarly maintained in Medium B. 100 ml of a Medium B subculture was used as inoculum for all the reactor experiments in which sterile Medium B (1,900 ml) was used.

As reported in a previous study (Armenante et al., 1992), facultative organisms were also present in the anaerobic culture in the reactor where degradation was observed. Therefore, the same enrichment culture that had been used to obtain the anaerobic inoculum was also used to isolate the aerobic enrichment culture.

4.2.3 Experimental Procedures

4.2.3.1 Experimental Procedures for the Anaerobic Systems

The acclimatization procedure consisted of transferring 4 ml of the original dehalogenating culture from Kung's reactor R3 (containing undefined medium) to two serum bottles containing a semi-defined medium (50% defined Medium A plus 50% undefined medium) to which 2,4,6-TCP and resazurin had been added. The final concentrations of 2,4,6-TCP and resazurin in the bottles were 100 μM and 0.0001%, respectively. The pH was 8.2. The bottles were incubated in the dark at 30 ^{o}C. Samples were taken by inserting a syringe needle through the butyl rubber stopper. Successive additions of 2,4,6-TCP were made by syringe injection through the rubber stopper. This experimental procedure was repeated with increasing percentages of defined medium (in 10% increments) up to 100% of defined medium. In all the experiments carried out in serum bottles, the inoculum were taken from the previous bottle.

A similar procedure was used in the experiments in serum bottles in which Medium B was used.

In the experiments designed to determine the effect of pH on dehalogenation the serum bottles contained defined Medium B plus one of the biological buffers shown in Table 6-2 (i.e., MOPS, TRICINE, BICINE, CHES). Defined Medium B plus the biological buffer, BICINE, were used in all the experiments designed to determine the effect of temperature and end-product concentration (4-CP) on the dehalogenation kinetics of 2,4,6-TCP.

For the anaerobic packed-bed reactor, 100 ml of anaerobic culture form serum bottles were transferred into the 2,000 ml liquid volume reactor containing 1,900 ml defined Medium B (to which 2,4,6-TCP and resazurin had been added) with BICINE, since the pH resulting from the use of this buffer was found to be the most appropriate for anaerobic dehalogenation to occur. The pH was 8.2. The reactor was incubated in the dark at 30 ^{o}C.

4.2.3.2 Experimental Procedures for the Aerobic Systems

In the Fernbach flask experiments 100 ml aliquots of the liquid content of the anaerobic recirculating immobilized batch reactor were transferred to duplicate flasks. This liquid contained only the 4-CP produced during the anaerobic dehalogenation process. Before transferring the liquid was analyzed so as to insure that no residual 2,4,6-TCP and 2,4-DCP remained.

The aerobic batch experiments were performed in either the flasks or the suspended cell reactor. A pink color was seen in the flasks, since resazurin had reacted with oxygen. Oxygen was supplied to the flasks by using loose culture plastic caps. Except for the pH study of aerobic mineralization in shake flasks, all the aerobic experiments were performed at 30 ^{o}C, pH 7.0 ~ 7.5, and in the dark. In the experiments designed to determine the pH effect the shake flasks contained defined Medium B plus one of the biological buffers shown in Table 6-2 (i.e., MOPS, BICINE, CHES).

A procedure similar to that described for the shake flask experiments was used for the experiment in the aerobic batch suspended cell reactor. In this case, however, 770 ml of liquid were transferred to the aerobic reactor and 70 ml of a 2.5 mM MOPS buffer solution were added.

The same reactor was also used in the continuous experiments. This reactor was continuously fed with the effluent from the anaerobic recirculating batch reactor and with a 2.5 mM MOPS solution. The flow rate of the stream from the anaerobic reactorwas in the range 0-190 ml/hr. The flow rate of the MOPS solution was 1/10 of the stream from the anaerobic reactor.

In the anaerobic-aerobic experiments the same aerobic reactor was continuously fed from the anaerobic recirculating continuous reactor.

In the batch and continuous suspended cell reactor experiments, oxygen was supplied by sparging air at 50 ml/min from the bottom of the reactor. The rate of 4-CP stripping from the reactor was determined by taking samples during experiments run under the same conditions but without bacteria. The rate of 4-CP removal via stripping was not significant, as reported in greater detail in the Results and Discussion chapter.

4.3 Analytical Chemicals, Materials and Equipment

4.3.1 Analytical Chemicals

Table 4-3 lists the chemicals used during the analytical part of this work.

Table 4-3 Chemical compounds used for analysis

Chemical Species	Supplier	Cat. Number	Note
2,6-DCP	Aldrich	D7020-1	
2-CP	Aldrich	18577-9	
3-CP	Aldrich	C6280-8	
Ionic Strength Reagent	Orion	94-00-11	required for $[Cl^-]$ measurement
10 % KNO_3	Orion	90-00-17	
Sodium Carbonate	Fisher	766181	
Sodium Potassium Tartrate	Fisher	770949	
Cupric Sulfate	Fisher	850092	
Clocalteu's Folin Phenol Reagent	Sigma	101H5027	
Methanol	Fisher	A452-2	HPLC Grade
Acetic Acid, Glacial	Baker	9515-03	HPLC Grade
Mini-q Water	American B. & J.	AP557	HPLC Grade
Hydrochloric Acid	RICCA	S284	6.00 N

4.3.2 Analytical Materials

The analytical materials are given in Table 4-4.

Table 4-4 Materials used for analytical methods

Materials	Supplier	Cat. Number	Note
Filter Paper	Gelman Science	66608	for HPLC solvents 47mm*0.45µm
Filter Paper	Millipore	GVHP 013 00	for HPLC samples 13mm*0.20µm
Plastic Filter Holders	Gelman Science	4317	for HPLC samples 13 mm
Sample Vials	Alltech	72710	for Waters 715 auto-sampler size: 4 ml
Sterile Centrifuge Tubes	Fisher	05-539-2	size: 15 ml
Borosilicate Culture Tubes	Fisher	14-962-10F	

4.3.3 Equipment

Table 4-5 gives the analytical equipment used in this work.

Table 4-5 Equipment used for analytical work

Manufacturer	Model Number	Purpose	Note
Waters	715 Ultra Wisp	auto-sampling process	For HPLC
Waters	600E	system controller	For HPLC
Waters	484 Tunable Absorbance Detector	UV detection	For HPLC
Alltech Econosphere	C8 5U	HPLC column, along with Refillable Guard Column	For HPLC
PE Nelson	2600	data acquisition unit (interface box)	For HPLC
PE Nelson	Version 5.10	chromatography software	For HPLC
Gilford	Stasar III	spectrophotometer	Folin Protein Assay
Orion	SA720	pH / ISE meter	
Orion	96-17B	chloride electrode	For Chloride Measur'nt
Orion	91-56	pH electrode	For pH Measur'nt

4.4 Analytical Methods

4.4.1 Determination of Chlorophenols (CPs) via HPLC

The samples taken from the serum bottles or reactors were stored at -15 ^{O}C. After thawing, all samples were centrifuged at 4500 rpm and 17 ^{O}C for 15 minutes. 2.0 ml of the supernatant of the centrifuged sample were acidified with 6.0 N HCl, aspirated into a syringe, and filtered through a filter holder containing the filter paper for High Performance Liquid Chromatography (HPLC) samples. The filtered sample was transferred to a standard auto-sampling vial, which was then placed in a Waters 715 Ultra Wisp sample processor. 25 µl (0.025 ml) of the sample were injected into an HPLC Waters system, consisting of a Waters 600E system controller and a Waters 484 Tunable Absorbance Detector.

An Alltech Econosphere C8 5U 4.6 mm (inner diameter) 150-mm length column was used. An Alltech Direct Connect Refillable Guard Column filled with Guard Column refills Pell for C8 was also used to protect the column. Mobil Phase A (1% acetic acid in methanol) and Mobil Phase B (1% acetic acid in Milli-q water), 60:40, was run isocratically at a flow rate equal to 1.0 ml/min. UV detection was set at 280 nm, 0.5 AUFS. The data were processed by the PE Nelson chromatography software, Ver. 5.10, interfaced with 760 Series Model 2600 data acquisition unit.

Calibration curves for the compounds of interest, i.e., 2-CP, 3-CP, 4-CP, 2,4-DCP, 2,6-DCP and 2,4,6-TCP, were obtained with standards of known concentration. The biodegradation products of 2,4,6-TCP were first identified by comparison with concentrations and HPLC retention times of known standards. The results were further confirmed by comparing the UV spectra of the dechlorination products with those of the standards.

4.4.2 Determination of Chloride Ion via Chloride Electrode

After thawing and centrifuging the sample, its chloride ion concentration [Cl^-] was determined with an Orion Model SA 720 pH/ISE meter and an Orion Model 96-17B chloride electrode at room temperature. Before the measurement, one drop of ionic strength reagent was added per 3 ml of sample volume, and the electrode was filled with a 10% KNO_3 solution. A calibration curve was obtained using chlorine solutions of known concentrations of the sample (between 3 ppm and 200 ppm). The chloride concentration of the sample was obtained by comparison with the standards. If necessary, the samples were diluted to obtain a millivolt response within the range of the instrument.

4.4.3 Determination of Biomass Concentration via Folin Protein Assay

The determination of biomass concentration was based on the Folin Protein Assay for bacterial suspension (which can be related to the dry-weight measurement in [mg/100 ml]). 1.0 ml of the sample was transferred to a plastic centrifugation tube and centrifuged at 3,500 rpm for 30 minutes at 4 ^{O}C. Then the supernatant of the sample was discarded. 1.0 ml of distilled water and 2.0 ml of 1.0 N NaOH were added to cell tubes, which were boiled at 90 ^{O}C for 10 minutes in a water bath. After cooling, Regent C (which was made from Reagent A and B described below) was added to the sample, and the sample was allowed to rest at room temperature for 10 minutes, 0.5 ml of Reagent D, and 1.0 N of Folin, were added to the sample, and the sample was allowed to rest for 30 minutes

An absorbance measurement was obtained from a Gilford Model Stasar III spectrophotometer, at 500 nm, and at room temperature. The absorbance was converted to dry-weight of biomass in [mg/100ml] using the calibration curve shown in Figure 4-8.

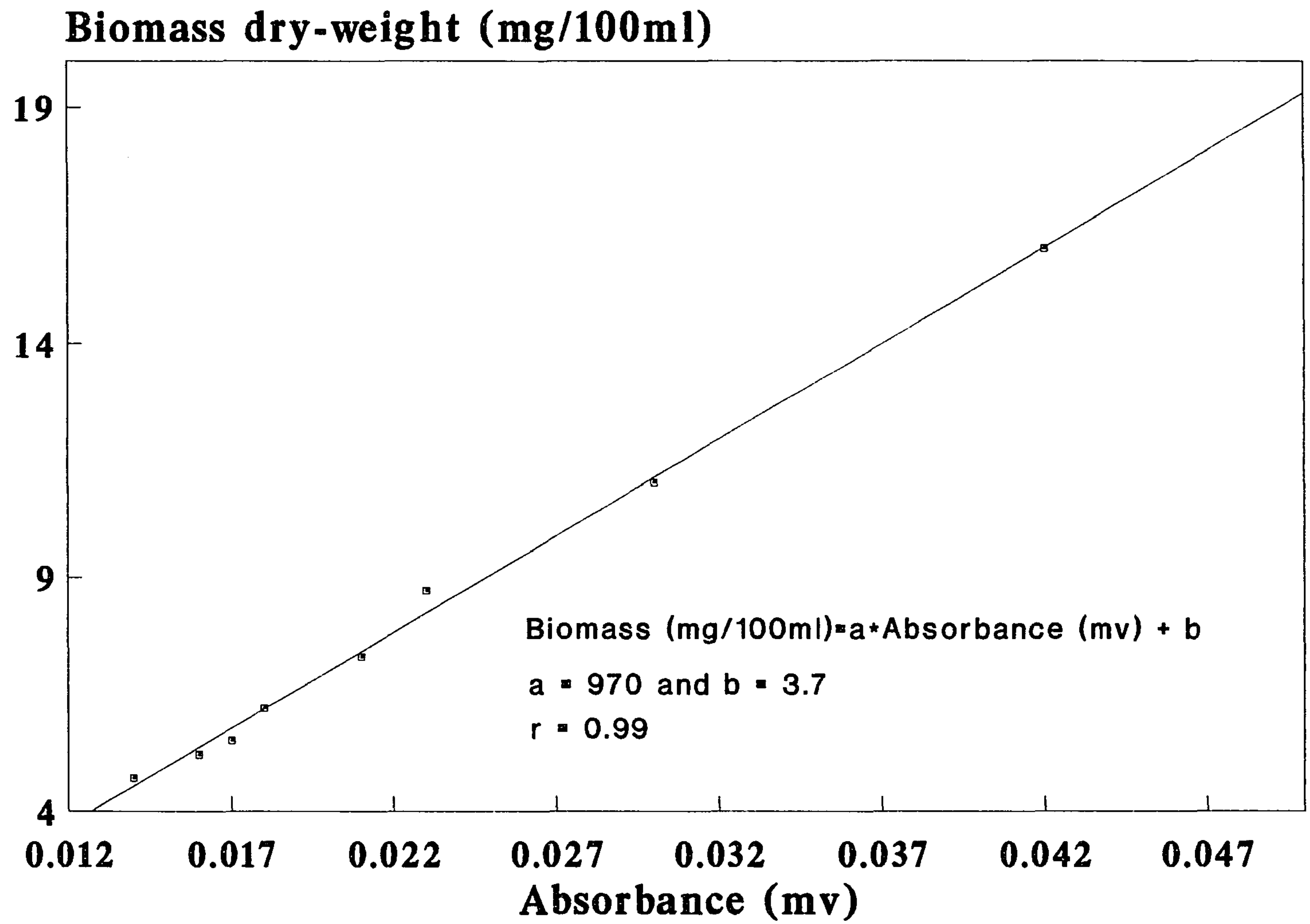

Figure 4-8 Calibration curve of protein assay for the measurement of biomass; absorbance in "mv" vs. biomass in "mg/100ml"

The compositions of Reagent A, B, C and D are as follows:

REAGENT A --- 20 g of Na_2CO_3 in one liter of distilled water

REAGENT B --- 0.5 g of $CuSO_4 \cdot 15H_2O$ in 100 ml of 1% (wt/vol.) aqueous solution of "Sodium Potassium Tartrate."

REAGENT C --- mixture of 50 ml of reagent A and 1 ml of reagent B. (Used for only one day)

REAGENT D --- 1.0 N of Folin

4.4.4. Determination of pH via pH Electrode

The combination of an Orion Model SA 720 pH/ISE meter, a pH electrode (Orion Model 91-56), and a BNC connector were used to determine pH. The pH of each sample was measured by immersing the pH electrode into the liquid solution at room temperature.

Chapter 5

MATHEMATICAL MODELING OF THE ANAEROBIC AND AEROBIC PROCESSES

5.1 Mathematical Modeling of the Anaerobic Batch Process for 2,4,6-TCP Dehalogenation

The dehalogenation curves for a typical batch anaerobic experiment in serum bottles are shown in Figure 5-1. As reported in previous work by Armenante et al. (1992 and 1993), this figure shows that after an initial lag time 2,4,6-TCP was degraded to 2,4-DCP which was in turn degraded to 4-CP, anaerobically, according to the following reaction:

$$\text{2,4,6-TCP} \longrightarrow \text{2,4-DCP} \longrightarrow \text{4-CP}$$

The following assumptions were made during the development of the model:

1. The concentration of the substrate (2,4,6-TCP) was in the range of 40~150 μM. A non-inhibitory (Michaelis-Menten) model is assumed to be valid in this concentration range.
2. The reactor is well-mixed.
3. The biomass concentration is constant in any given system.

Then, the results for the experiments in the batch systems (serum bottles, anaerobic batch packed-bed reactors) can be analyzed using the following unsteady state mole balance equations

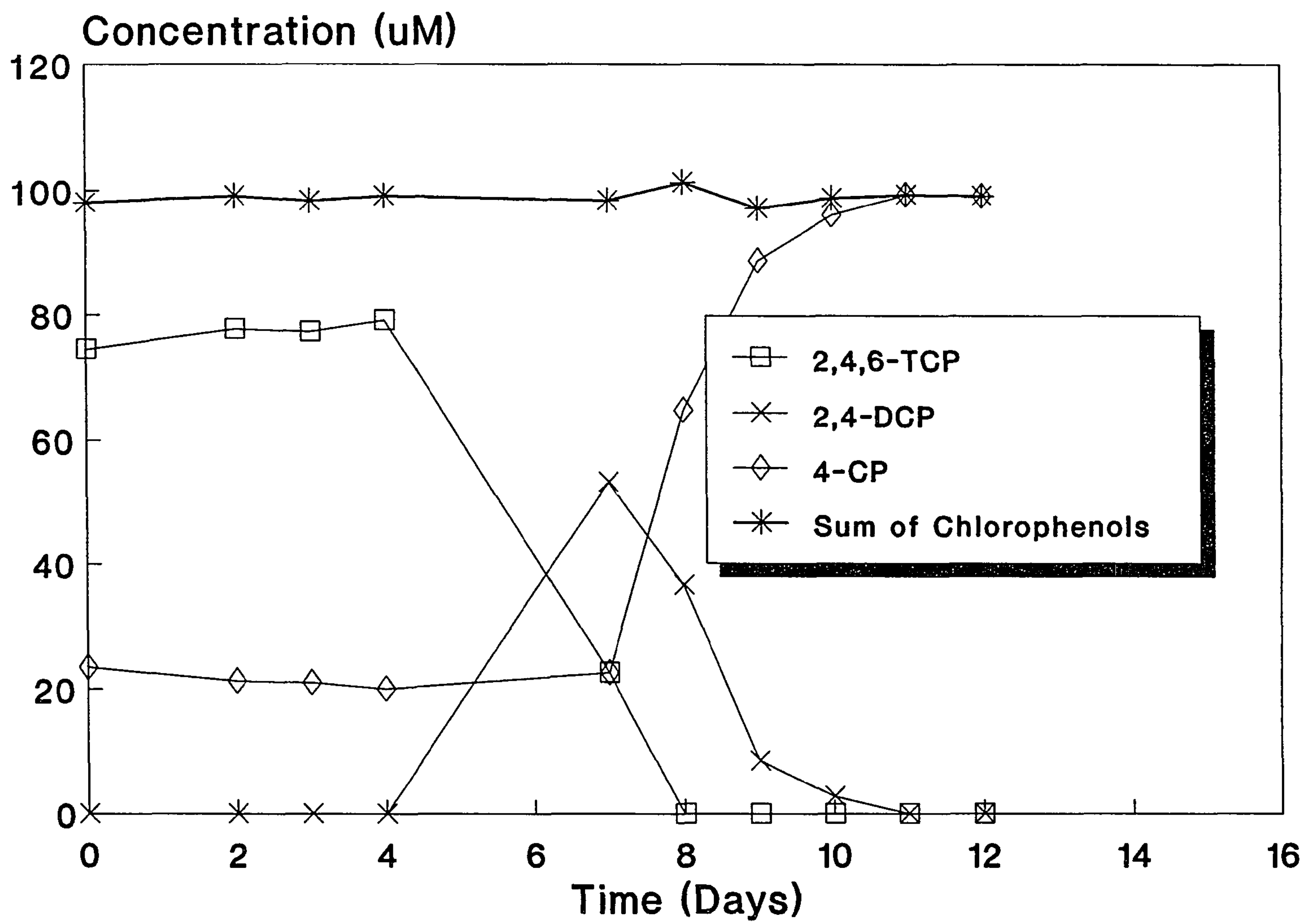

Figure 5-1 Anaerobic dechlorination of 2,4,6-TCP in a serum bottle using undefined medium (after Kung (1991))

$$\frac{dC_{TCP}}{dt} = -\frac{Ka_{TCP} \cdot C_{TCP}}{Kb_{TCP} + C_{TCP}} \tag{5.1}$$

$$\frac{dC_{DCP}}{dt} = \frac{Ka_{TCP} \cdot C_{TCP}}{Kb_{TCP} + C_{TCP}} - \frac{Ka_{DCP} \cdot C_{DCP}}{Kb_{DCP} + C_{DCP}} \tag{5.2}$$

$$\frac{dC_{CP}}{dt} = \frac{Ka_{DCP} \cdot C_{DCP}}{Kb_{DCP} + C_{DCP}} \tag{5.3}$$

where

C_{TCP} = Concentration of 2,4,6-TCP (μM)

C_{DCP} = Concentration of 2,4-DCP (μM)

C_{CP}: = Concentration of 4-CP (μM)

t = Time (hr)

Ka_{TCP}, Ka_{DCP} = Apparent Michaelis-Menten proportionality constants for 2,4,6-TCP and 2,4-DCP dehalogenation (μM/hr)

Kb_{TCP}, Kb_{DCP} = Michaelis-Menten half-saturation constants for 2,4,6-TCP and 2,4-DCP dehalogenation (μM)

5.2 Mathematical Modeling of the Immobilized Recirculating Continuous Anaerobic Reactors for 2,4,6-TCP and 2,4-DCP Dehalogenation

The equations for the continuous system were derived under the same assumptions made in the derivation of the model for the anaerobic batch process, and assuming that the reactor is well mixed because of the presence of the recirculation flow.

For the anaerobic continuous packed-bed recirculating reactor the corresponding mole balance equations are

$$QC_{TCPin} - QC_{TCP} = (-r_{TCP}) \cdot V \tag{5.4}$$

$$QC_{DCPin} - QC_{DCP} = (-r_{DCP}) \cdot V \tag{5.5}$$

$$QC_{CPin} - QC_{CP} = (-r_{CP}) \cdot V \tag{5.6}$$

where

$$r_{TCP} = -\frac{Ka_{TCP} \cdot C_{TCP}}{Kb_{TCP} + C_{TCP}} \tag{5.7}$$

$$r_{DCP} = \frac{Ka_{TCP} \cdot C_{TCP}}{Kb_{TCP} + C_{TCP}} - \frac{Ka_{DCP} \cdot C_{DCP}}{Kb_{DCP} + C_{DCP}} \tag{5.8}$$

$$r_{CP} = \frac{Ka_{DCP} \cdot C_{DCP}}{Kb_{DCP} + C_{DCP}} \tag{5.9}$$

where

C_{TCPin} = Input concentration of 2,4,6-TCP (μM)

C_{DCPin} = Input concentration of 2,4-DCP (μM)

C_{CPin} = Input concentration of 4-CP (μM)

C_{TCP} = Output concentration of 2,4,6-TCP (μM)

C_{DCP} = Output concentration of 2,4-DCP (μM)

C_{CP} = Output concentration of 4-CP (μM)

Q = Volumetric flow rate (ml/hr)

V = Anaerobic reactor operating volume (ml)

5.3 Mathematical Modeling of the Anaerobic Batch Process for 2,4-DCP Dehalogenation

In those experiments in which 2,4-DCP was the sole halogenated compound to be degraded the reaction mechanism was assumed to be

2,4-DCP ———> 4-CP

Similar assumptions to those made for the previous models were made, i. e.,:

1. The concentration of the substrate (2,4-DCP) was in the range of 40~80 µM. A non-inhibitory (Michaelis-Menten) model was assumed to be valid in this concentration range.
2. The reactor is well-mixed.
3. The biomass concentration is constant in any given system.

The resulting kinetic expression is

$$\frac{dC_{DCP}}{dt} = -\frac{Ka_{DCP} \cdot C_{DCP}}{Kb_{DCP} + C_{DCP}} \tag{5.10}$$

$$\frac{dC_{CP}}{dt} = \frac{Ka_{DCP} \cdot C_{DCP}}{Kb_{DCP} + C_{DCP}} \tag{5.3}$$

5.4 Mathematical Modeling of the Aerobic Batch and Continuous Processes for 4-CP Mineralization

Equations similar to those given for the anaerobic systems can also be written for the aerobic processes provided that a similar set of assumptions as that made for anaerobic systems holds true. In the aerobic case the biomass was assumed to be constant since experiments (whose results are given in the next chapter) indicated that this was indeed the case. The basic aerobic mineralization reaction takes the form

4-CP ———> Mineralization Products

Then, the results for the experiments in the aerobic batch system (shake flasks) can be analyzed using the following unsteady state mole balance equation

$$\frac{dC_{CP}}{dt} = -\frac{Ka_{CP} \cdot C_{CP}}{Kb_{CP} + C_{CP}} \tag{5.11}$$

and those for continuous reactor using the equations

$$QC'_{CPin} - QC'_{CP} = (r'_{CP}) \cdot V' \tag{5.12}$$

$$r'_{CP} = -\frac{Ka_{CP} \cdot C'_{CP}}{Kb_{CP} + C'_{CP}} \tag{5.13}$$

where

C'_{CPin} = Input concentration of 4-CP (μM)

C'_{CP} = Output concentration of 4-CP (μM)

V' = Aerobic reactor operating volume (ml)

Ka_{CP} = Apparent Michaelis-Menten proportionality constant for 4-CP mineralization (μM/hr)

Kb_{CP} = Michaelis-Menten half-saturation constant for 4-CP mineralization (μM)

and where the prime size indicated that the 4-CP mineralization process is occurring in the aerobic system.

5.5 Mathematical Modeling of the Anaerobic-Aerobic Batch and Continuous Processes

Since the aerobic process did not result in any attack on the 2,4,6-TCP and 2,4-DCP molecules, the concentrations of these two species at the end of anaerobic-aerobic process were identical to those found at the end of the anaerobic process. Hence, the concentrations of 2,4,6-TCP and 2,4-DCP at the end of the combined continuous process were predicted using Equation 5.4 and 5.5.

The final 4-CP concentration at the end of the anaerobic-aerobic process was predicted using Equation 5.12 (for aerobic process) in which the concentration C'_{cp} (indicating the 4-CP concentration in the inlet to aerobic reactor) was replaced with the term C_{cp} in Equation 5.6 (indicating the concentration in the outlet of the anaerobic reactor). The final equation for C'_{cp} is therefore:

$$C'_{CP} = C_{CP}\frac{V}{Q} + r_{CP} - r'_{CP}\frac{V'}{Q} \qquad (5.14)$$

5.6 Modeling of the Temperature Effect on the Anaerobic Dehalogenation of 2,4,6-TCP

In order to estimate the temperature effect on the anaerobic dehalogenation process (the most critical in the anaerobic-aerobic process), the results of the experiments conducted at different temperature were interpreted using the following equations (Topiwala and Sinclair, 1971):

$$Ka_{TCP} = Ka_{0TCP} \exp(-\frac{E_{TCP}}{RT}) - Ka'_{0TCP} \exp(-\frac{E'_{TCP}}{RT}) \qquad (5.15)$$

$$Ka_{DCP} = Ka_{0DCP} \exp(-\frac{E_{DCP}}{RT}) - Ka'_{0DCP} \exp(-\frac{E'_{DCP}}{RT}) \qquad (5.16)$$

In the temperature range 19 - 30 ^{o}C, it was assumed that the second terms of the right-hand-side of Equations 5.15 and 5.16 are negligible. Therefore, the above equations become:

$$Ka_{TCP} = Ka_{0TCP} \exp(-\frac{E_{TCP}}{RT}) \qquad (5.17)$$

and

$$Ka_{DCP} = Ka_{0DCP} \exp(-\frac{E_{DCP}}{RT}) \qquad (5.18)$$

The use of these equations implies that the temperature is assumed to have a significant effect on Ka_{TCP} and Ka_{DCP} but only a negligible effect on Kb_{TCP} Kb_{DCP}. However, this approach was chosen since only a limited number of experimental points in which the temperature was changed was available. On the other hand, this approach can indicate whether the orders of magnitude for the regressed values for E_{TCP} and E_{DCP} are correct or not.

Chapter 6
RESULTS AND DISCUSSION

6.1 Anaerobic Process

6.1.1 Anaerobic Dehalogenation During the Experiments Focused on the Development of Defined Media

Figure 6-1 shows the results for the experiments conducted in the presence of Medium A (Table 6-1), after the acclimation to the synthetic medium was successfully completed. 2,4,6-TCP was completely and stoichiometrically converted to 2,4-DCP and eventually 4-CP within 4 days, following a 4-day incubation period. The lag phase disappeared when the system was additionally spiked with 2,4,6-TCP. The initial concentration of 4-CP was different from zero because the inoculum from the previous bottle contained 4-CP as a residual product of the reductive dechlorination of 2,4,6-TCP. When pure Medium A was used, the pH was measured only at the end of the dechlorination process and was found to be around 8.1. Similar dechlorination patterns were obtained also when semi-synthetic media were used. However, in this case the final pH was typically higher (about 9.1), and a decrease in the dehalogenation activity was observed. Although further dechlorination activity was not studied once all the more highly chlorinated chlorophenols were converted to 4-CP the results of this work preliminary suggests that anaerobic dehalogenation did not proceed appreciably after the highly chlorinated chlorophenols were converted to monochlorophenols. This observation is in line with the results of previous studies (Armenante et al., 1992; Kafkewitz et al., 1992; Madsen and Licht, 1992; Mohn and Kennedy, 1992b).

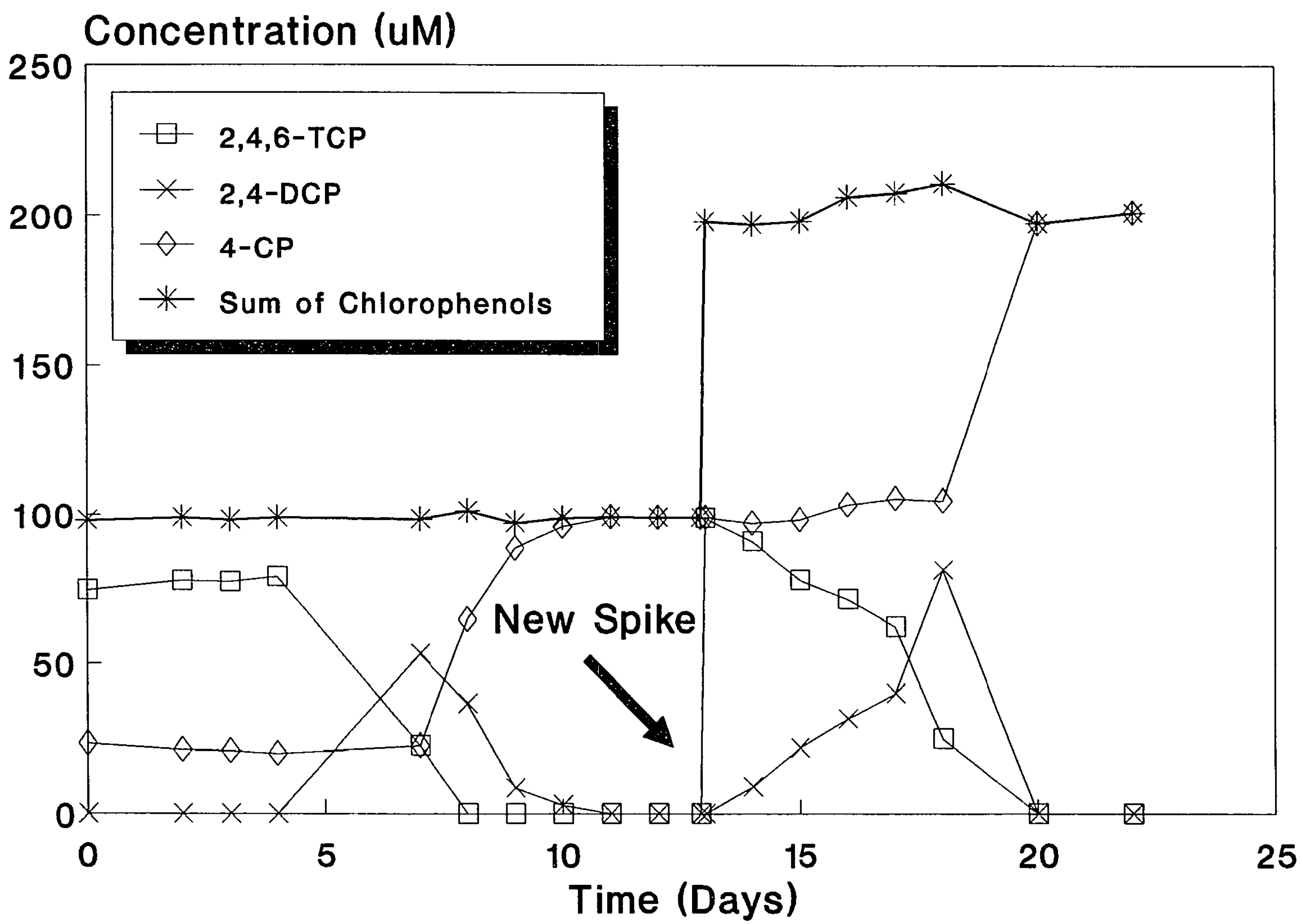

Figure 6-1 Anaerobic dechlorination of 2,4,6-TCP in a serum bottle using Medium A

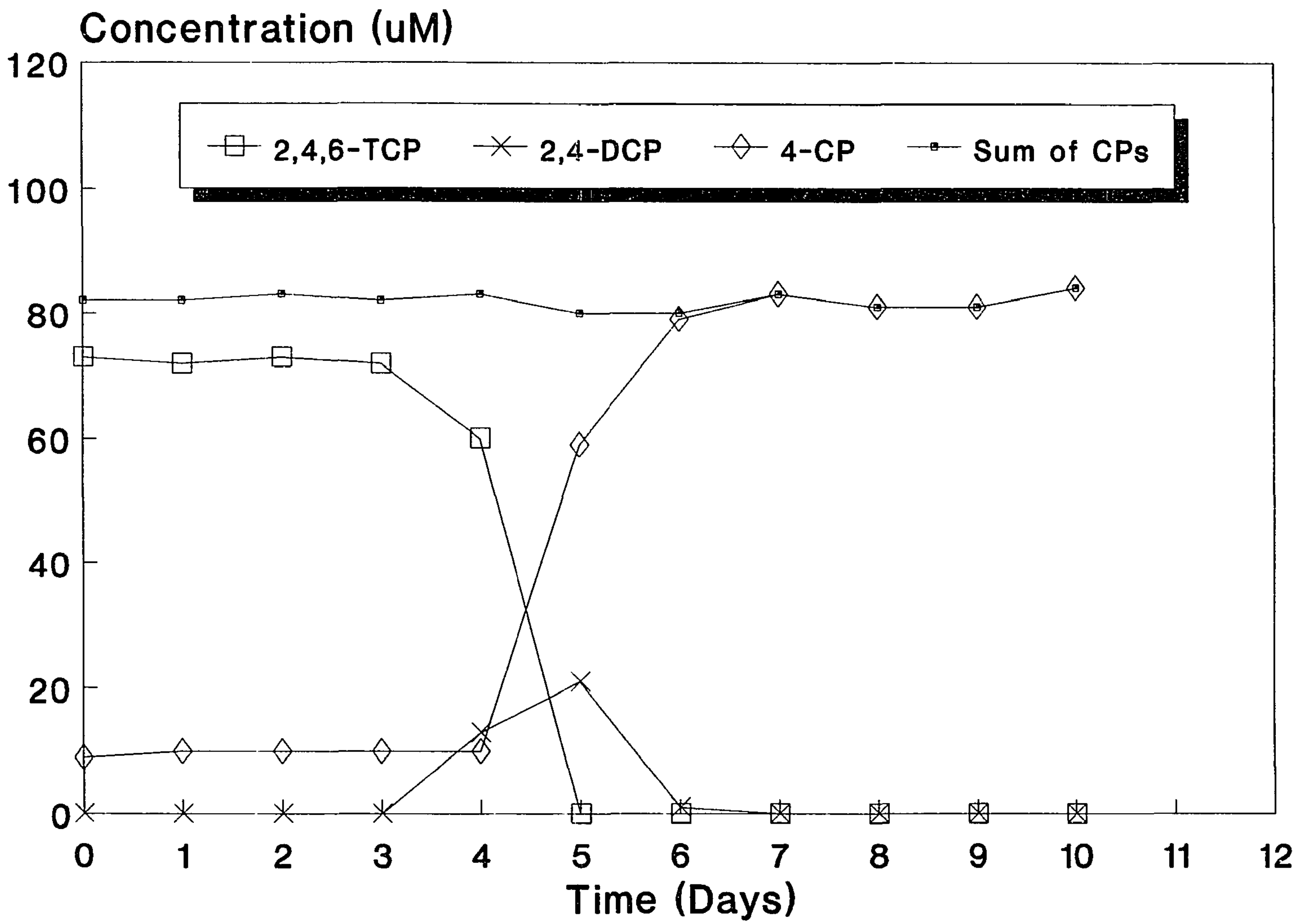

Figure 6-2 Anaerobic dechlorination of 2,4,6-TCP in a serum bottle using Medium B

Table 6-1 Compositions of the defined media used in this work

Medium A		Medium B	
Chemical Species	Concentration (g/liter)	Chemical Species	Concentration (g/liter)
KH_2PO_4	0.45	KH_2PO_4	0.45
K_2HPO_4	0.45	K_2HPO_4	0.225
$MgSO_4{\cdot}7H_2O$	0.18	$MgSO_4{\cdot}7H_2O$	0.09
$CaCl_2{\cdot}2H_2O$	0.012	$FeSO_4{\cdot}7H_2O$	0.002
NH_4Cl	1.0	$(NH_4)_2SO_4$	0.20
Resazurin	0.001	Resazurin	0.001
--	--	$NaHCO_3$	2.5
Sodium Formate	2.0	Sodium Formate	2.0
Sodium Acetate	2.5	Sodium Acetate	2.5
Succinic Acid	2.0	Non-Fermentable Buffering Agent	See Table 2

Figure 6-2 and Figure 6-3 show the typical pattern of dechlorination in the experiments conducted in the presence of Medium B (Table 6-1), after the acclimation to the synthetic medium was successfully completed. Figure 6-2 and Figure 6-3 show that dechlorination began after a 3-day incubation period, and resulted in the complete disappearance of 2,4,6-TCP within the following 3 days. The experiment of Figure 6-2 was performed in a serum bottle, and that of Figure 6-3 in an immobilized recirculating batch reactor. In both cases the pH was buffered at 8.2 using BICINE as the buffering agent (Table 6-2). These results also confirm that anaerobic dehalogenation ceased when 2,4,6-TCP was converted to 4-CP within a period of 10 days.

6.1.2 Effect of pH on the Anaerobic Dehalogenation of 2,4,6-TCP

The effect of pH on dehalogenation is shown in Figure 6-4. In these experiments Medium B and the buffering agents (Table 6-2) were used. It is clear from this figure that dehalogenation is sensitive to pH and that this effect is independent of the presence of phosphate. In addition, this figure indicates that the pH range in which the dehalogenation process is able to be carried out is between 8.0 and 8.8. These results are in line with the preliminary conclusions drawn from the earlier work (Armenante et al., 1992; Kafkewitz et al., 1992). All these findings show that an operating range for dehalogenation exists, the definition of which can be of significant importance during the operation of any process based on this degradation approach.

The dehalogenation in the pH range given above (buffered by BICINE) was explored in greater detail in an additional experiment the results of which are given in Figures 6-5, 6-6, and 6-7, and in Table 6-3. A lag phase can still be observed in Figure 6-5 although only 2 days long, as opposed to 4 days as in Figure 6-1. If the lag phase was discounted the entire dehalogenation process

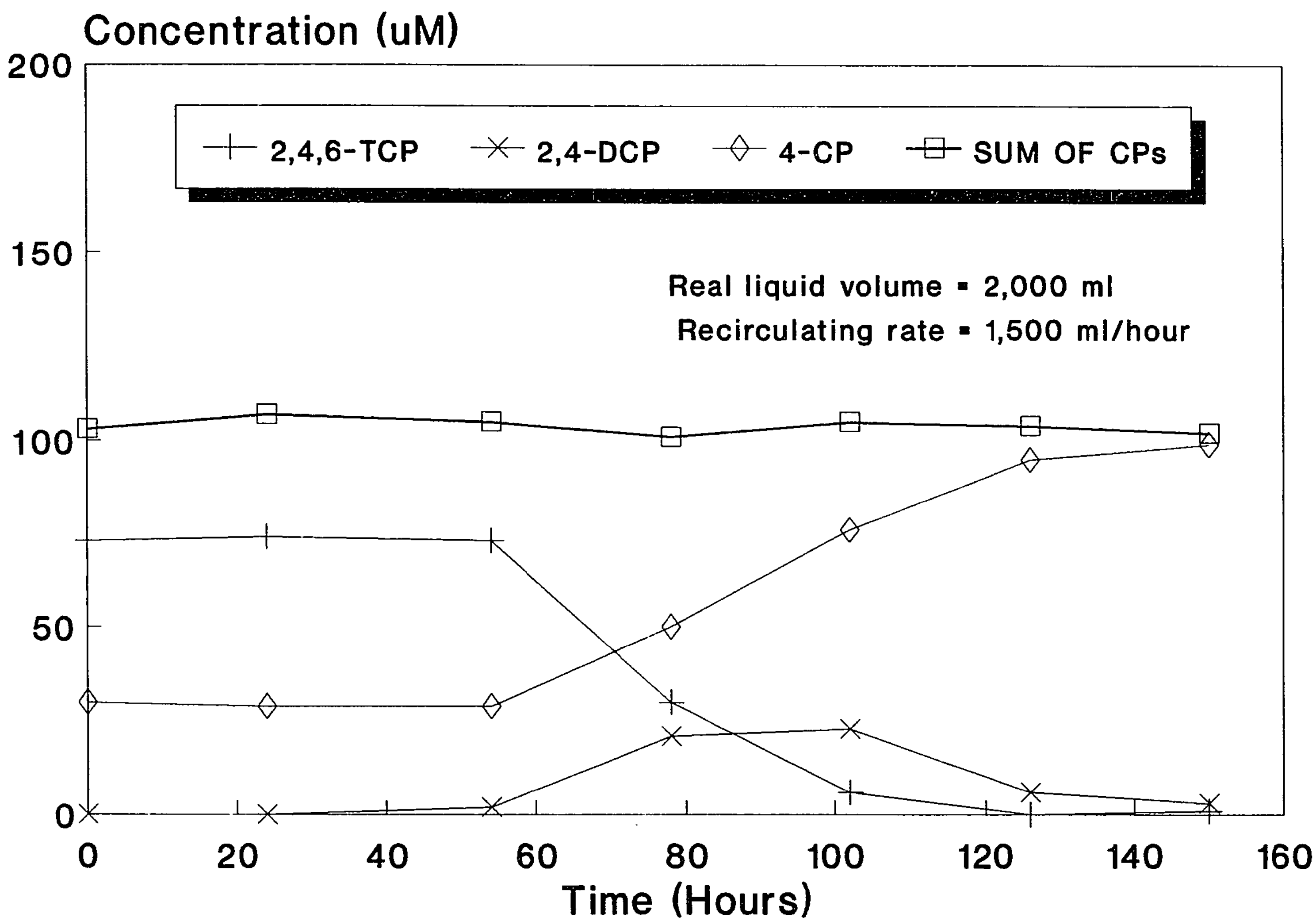

Figure 6-3 Anaerobic dechlorination of 2,4,6-TCP in an immobilized recirculating batch reactor using Medium B

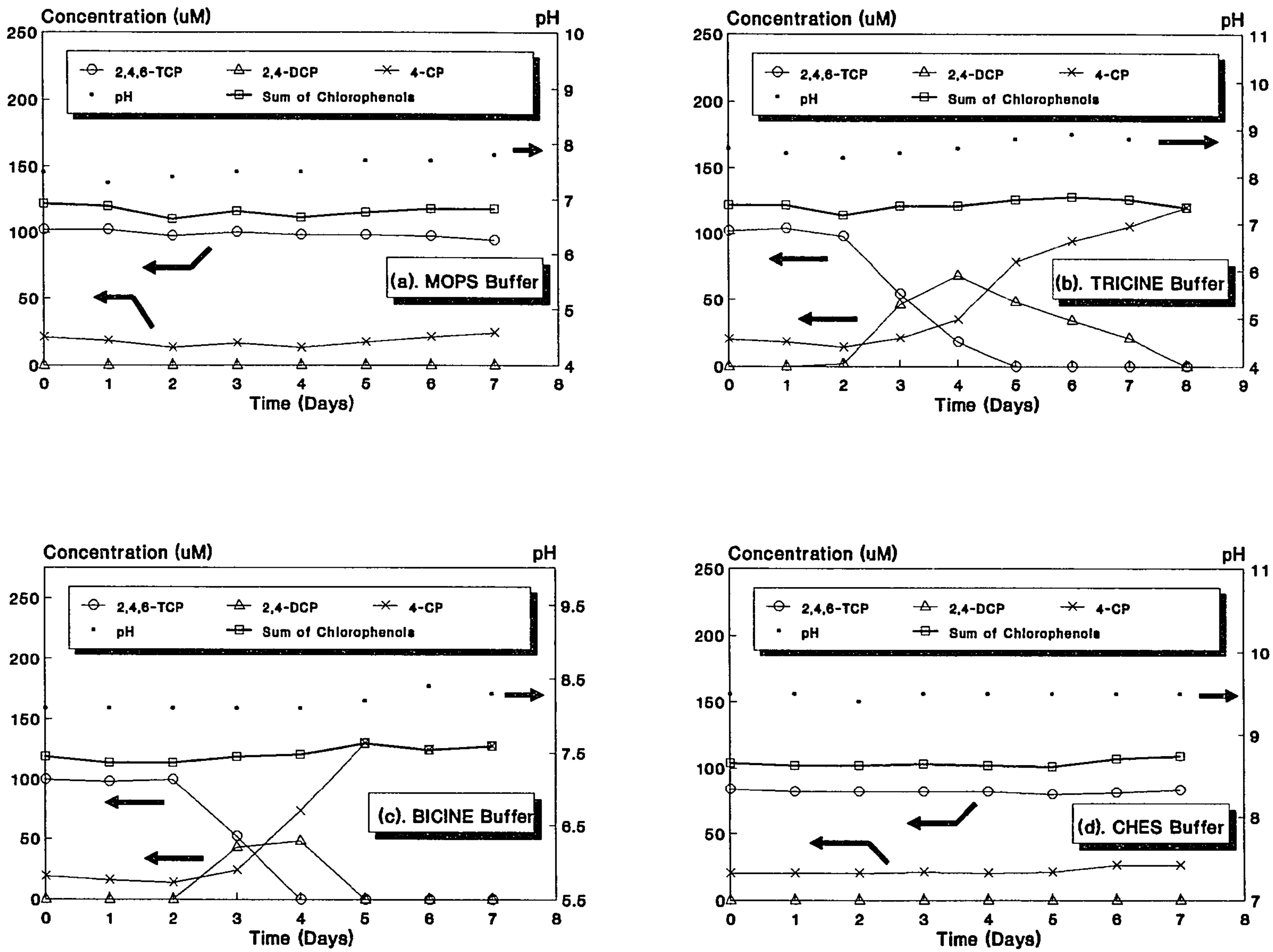

Figure 6-4 Effect of pH on dechlorination of 2,4,6-TCP

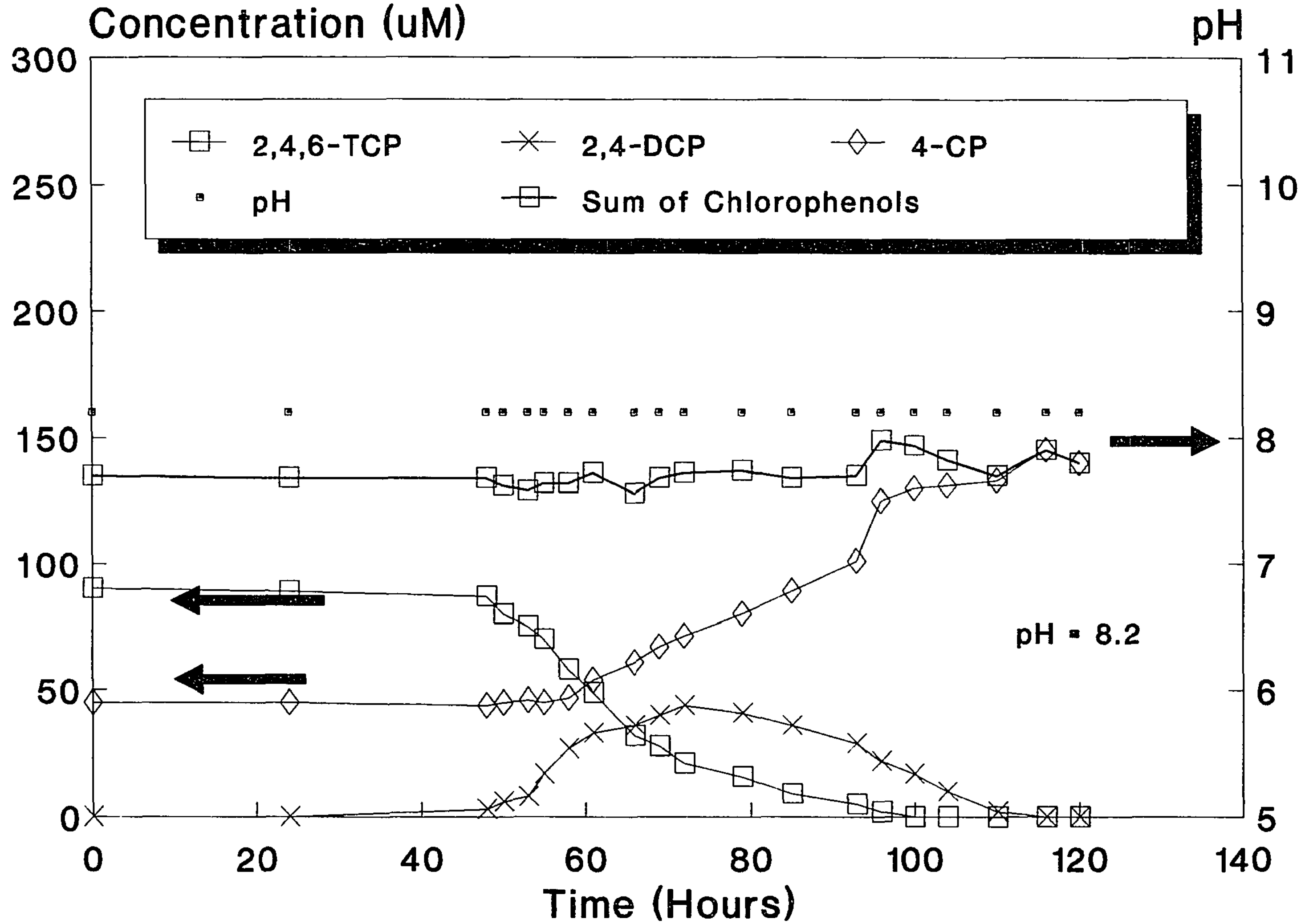

Figure 6-5 Anaerobic dechlorination of 2,4,6-TCP in Medium B containing BICINE

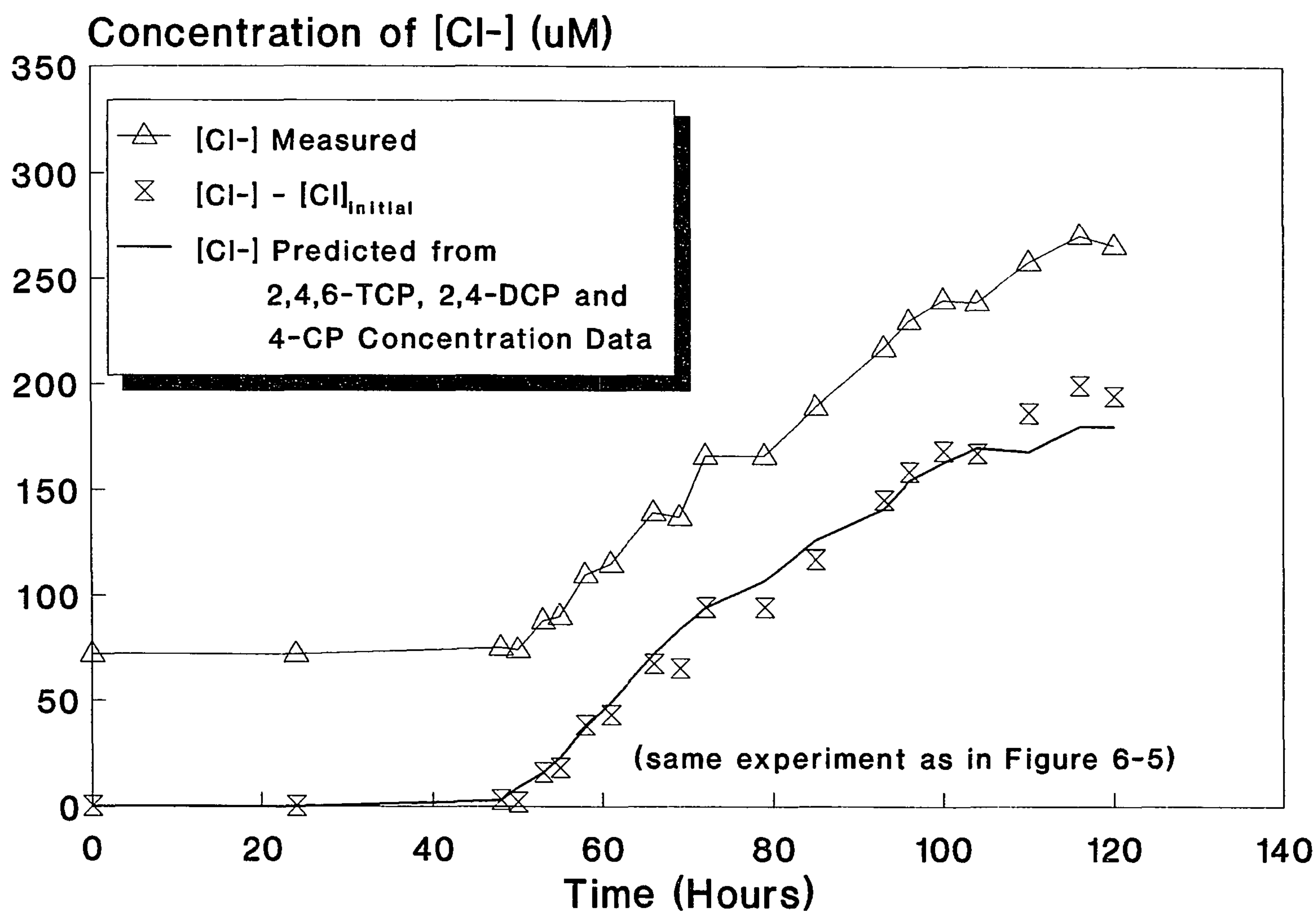

Figure 6-6 [Cl-] recovery during anaerobic dechlorination of 2,4,6-TCP

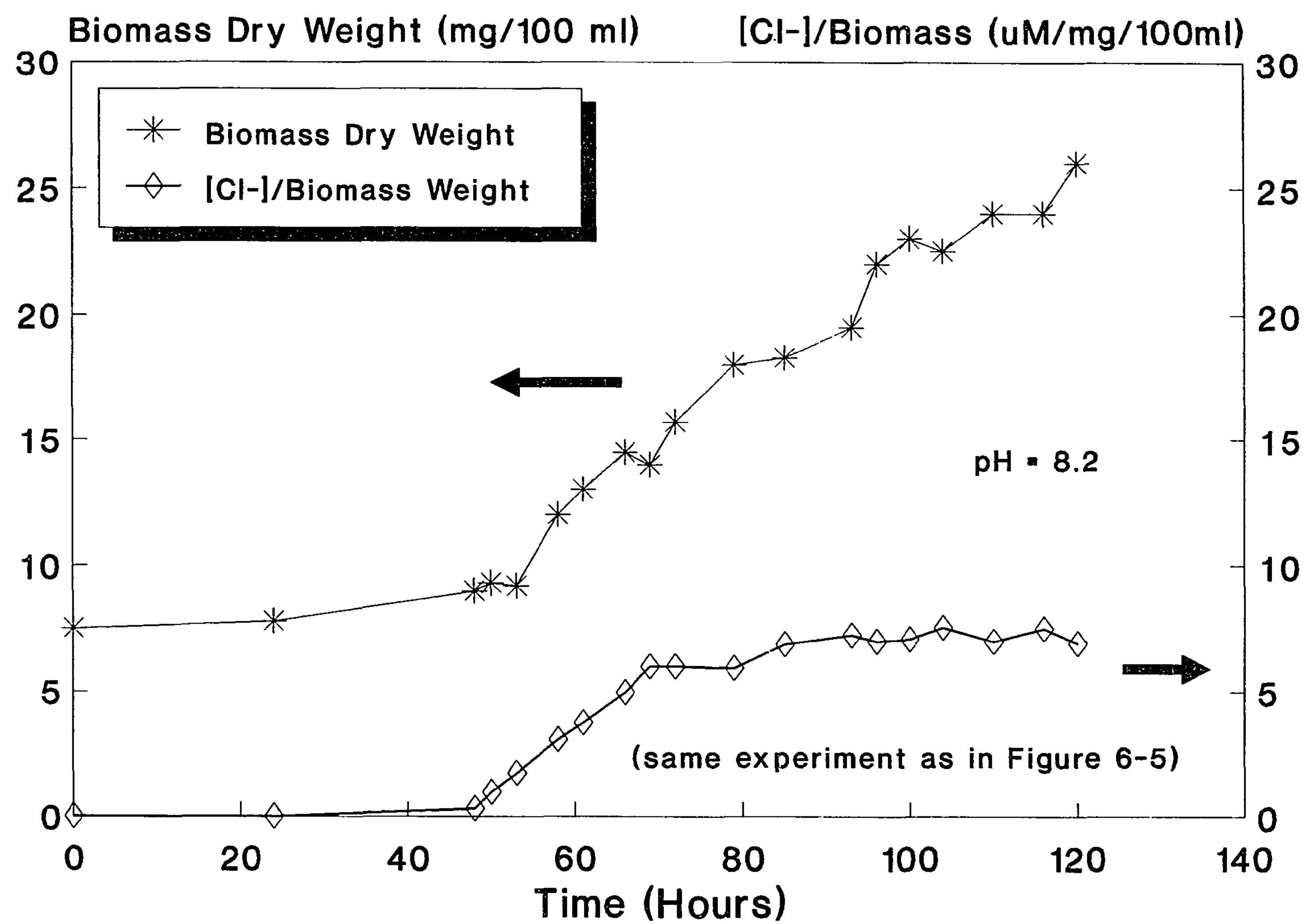

Figure 6-7 Biomass growth rate during anaerobic dechlorination of 2,4,6-TCP

Table 6-2 Non-fermentable buffering agents used in this work

Buffering Agent	pK_a	pH Range	Concentration in Medium B (mM)	Initial pH of Medium B
MOPS	7.2	6.5 ~ 7.9	45	7.4
TRICINE	8.1	7.4 ~ 8.8	20	8.6
BICINE	8.3	7.6 ~ 9.0	30	8.1
CHES	9.3	8.6 ~ 10.0	30	9.5

Table 6-3 Concentration, of 2,4,6-TCP, 2,4-DCP and 4-CP, and chloride ion during the dechlorination of 2,4,6-TCP in a serum bottle using Medium B, inculding biomass dry-weight

Time (hr)	pH	2,4,6-TCP (µM) Expt.	2,4-DCP (µM) Expt.	4-CP (µM) Expt.	total CPs (µM)	CPs % Reco	Cl^- (µM) Expt.	Cl^- diff. (µM) Expt.	Biom mg/ 100ml Expt.
0	8.2	90	0	45	135	100	72	0	7.5
24	8.2	89	0	45	134	99	72	0	7.8
48	8.2	87	3	44	134	99	75	3	9.0
50	8.2	80	6	45	131	97	74	2	9.3
53	8.2	75	8	46	129	96	88	16	9.2
55	8.2	70	17	45	132	98	90	18	/
58	8.2	58	27	47	132	98	110	38	12.0
61	8.2	49	33	54	136	101	115	43	13.0
66	8.2	32	36	60	128	95	139	67	14.5
69	8.2	28	40	66	134	99	137	65	14.0
72	8.2	21	44	71	136	101	166	94	15.7
79	8.2	16	41	80	137	101	166	94	18.0
85	8.2	9	36	89	134	99	189	117	18.3
93	8.2	5	29	101	135	100	217	145	19.5
96	8.2	2	22	125	149	110	230	158	22.0
100	8.2	0	17	130	147	109	240	168	23.0
104	8.2	0	10	131	141	104	239	167	22.5
110	8.2	0	2	133	135	100	258	186	24.0
116	8.2	0	0	145	145	107	271	199	24.0
120	8.2	0	0	140	140	104	266	194	26.0

*** A 125 ml serum bottle was used.**
The temperature was 30 °C

took place in less than 3 days, as opposed to 7. This faster process could be caused by a better acclimation of the organisms to the medium or by a larger amount of biomass initially present in the inoculum. In any case one can see that the conversion of 2,4,6-TCP to 2,4-DCP and 4-CP is stoichiometric. Quantitative recovery during anaerobic dehalogenation has also been recently reported by other investigators (Nicholson et al., 1992).

Since Medium B was designed to contain no chloride it was possible to confirm the stoichiometry of dehalogenation by measuring the change in chloride concentration and hence the amount of chloride produced. The initial concentration of chloride was not zero because the inoculum contained some residual chloride as one of the dechlorination products from the previous experiment. Table 6-3 reports the experimentally determined chloride concentration as well as the difference between the concentration of chloride ion at a generic time and that at time $t = 0$, shown in the table. This concentration difference is directly proportional to the amount of chloride ion produced during the dechlorination process. From Figure 6-6 one can see that this concentration matched very closely the concentration of chloride ion that one would predict from the concentrations of 2,4,6-TCP, 2,4-DCP and 4-CP in Figure 6-5 assuming stoichiometric dechlorination (solid coarse line).

Figure 6-7 shows that the biomass concentration (expressed as weight of dry biomass, in mg, per 100 ml of medium) increased significantly during the process. This figure also gives the ratio of chloride concentration to biomass concentration. Following the initial lag phase, the chloride concentration increased during the exponential growth phase between $t \approx 50$ hr and $t \approx 70$ hr. The ratio then stabilized after 70 hours, indicating that the rate of dechlorination per unit biomass was approximately constant. This, in turn, indicated that the dechlorinating organism(s) are either making up the entire population or constitute a stable fraction of it.

Microscopic observation of the cultures indicated the presence of at least three organisms: a large vibrio, a smaller vibrio, and an occasional long filamentous cell. Methane determination showed only traces of methane in the head space of serum bottles and reactors. From the available data it is not clear whether the dehalogenation was performed by a single organism or by the interactions of several organisms.

The dehalogenating cultures are relatively resistant to oxidative stress. Cultures exposed to enough oxygen to convert the resazurin to its pink oxidized form were not killed and did not lose their dehalogenating activity even when the serum bottle was exposed to oxygen (the medium remained pink) for as long as 48 hours. When the cultures were provided with substrates, they were capable of reducing the media sufficiently to make the serum bottle with resazurin colorless.

The aerobic mineralization ability against 4-CP reported in earlier studies by Armenante et al. (1992) and Kafkewitz et al. (1992) was lost after the cultures were transferred to Defined Media A or B.

As described above, the effective pH range for anaerobic dehalogenation of 2,4,6-TCP was 8.0 ~ 8.8. When the culture pH was 9.0 to 9.5, dehalogenation did not occur; however, the culture was not killed and the anaerobic dehalogenating activity resumed when the culture was buffered to pH 8.2 after as much as seven days at high pH. Similar behavior was observed for the lower pH range (from 6.8 to 7.8): the cultures were also able to degrade 2,4,6-TCP when the medium was buffered at pH = 8.2 after as much as seven days at low pH. The typical lag time for dehalogenation to occur after the medium was properly buffered was 2 days.

6.1.3 Results for the 2,4,6-TCP Dehalogenation Experiments and Their Interpretation Using the Proposed Model

The data taken from Table 6-3 were analyzed using Equations 5.1, 5.2, and 5.3. The results are shown in Figure 6-8. Ka_{TCP} and Kb_{TCP} were obtained by a linear regression of the integral of Equation 5.1. Ka_{DCP} and Kb_{DCP} were obtained by integrating Equations 5.2 and 5.3 using a fourth-order Runge-Kutta routine, and incrementally changing the values (in units of 0.05 μM/hr for Ka_{DCP}, and 0.01 μM for Kb_{DCP}) until the sum of the absolute error of the concentration was minimized. The difference between 0.05 and 0.01 increments was negligible in terms of absolute error. The values for the Michaelis-Menten constants, Ka_{TCP}, Kb_{TCP}, Ka_{DCP}, and Kb_{DCP}, are 3.2 μM/hr, 6.6 μM, 1.6 μ M/hr, and 1.9 μM, respectively. In Figure 6-8 the curves obtained with this method are compared with the experimental results (also given in Table 6-3).

Figure 6-9 shows a similar plot for another serum bottle containing a different amount of inoculum. The experimental data are taken from Table 6-4. The curves shown in this figure were obtained by keeping unchanged the values of Kb_{TCP} and Kb_{DCP} obtained previously, since those constants are not, in principle, a function of biomass (However, it should be pointed out that actual values of the Kb's obtained from the regression of the data could be slightly affected by the curve fitting process in which no biomass term is present). Ka_{TCP} and Ka_{DCP} were varied until the sum of the absolute error was minimized. The result was 2.4 μM/hr and 1.2 μM/hr, respectively. Those values are comparable to those found in Figure 6-8. A sensitivity analysis showed no significant difference (less than 7%) in the sum of the absolute error due to the differences in Ka_{TCP} and Ka_{DCP} (Table 6-5).

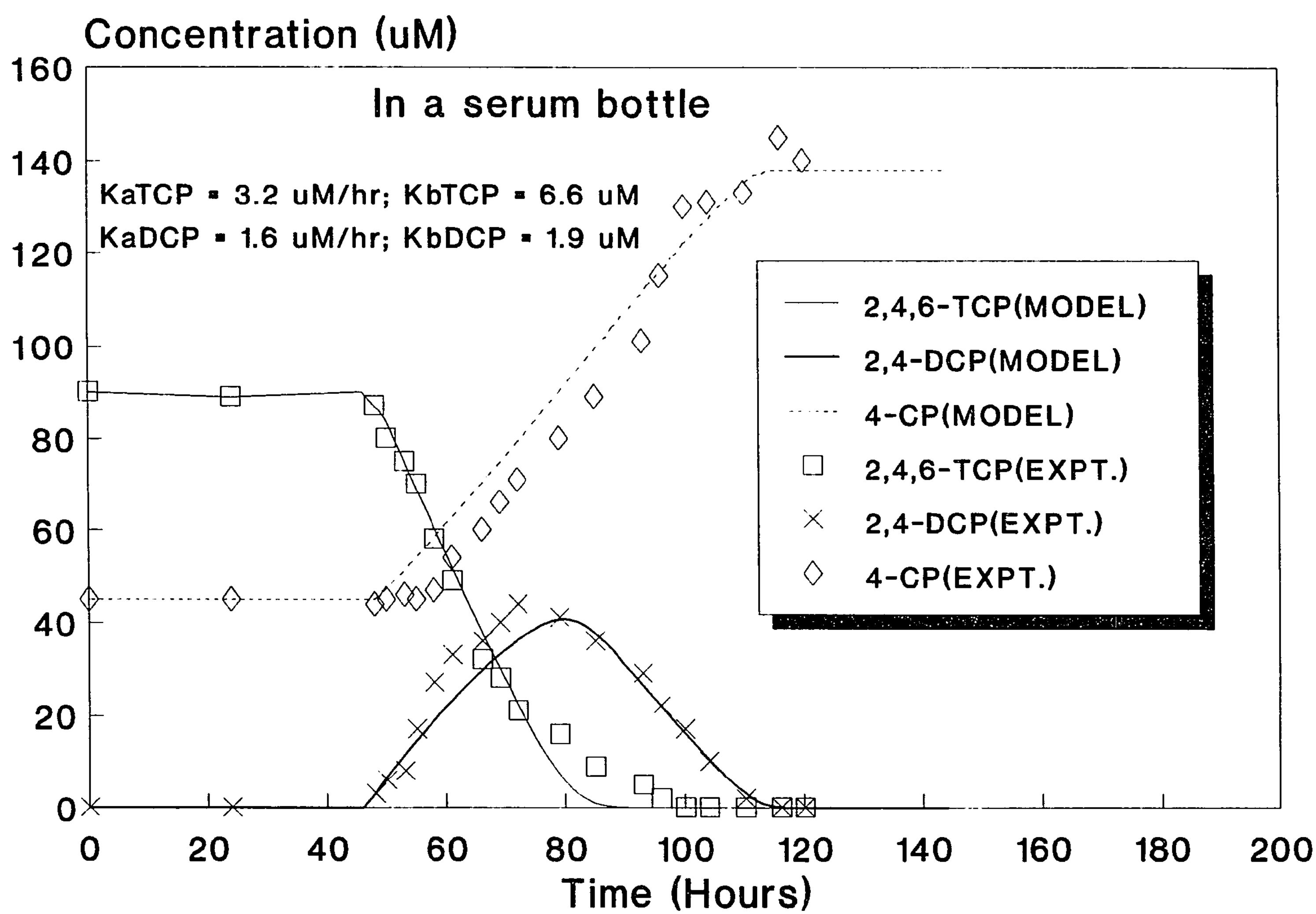

Figure 6-8 Experimental data and fitted curves for the anaerobic dehalogenation of 2,4,6-TCP (No. 1)

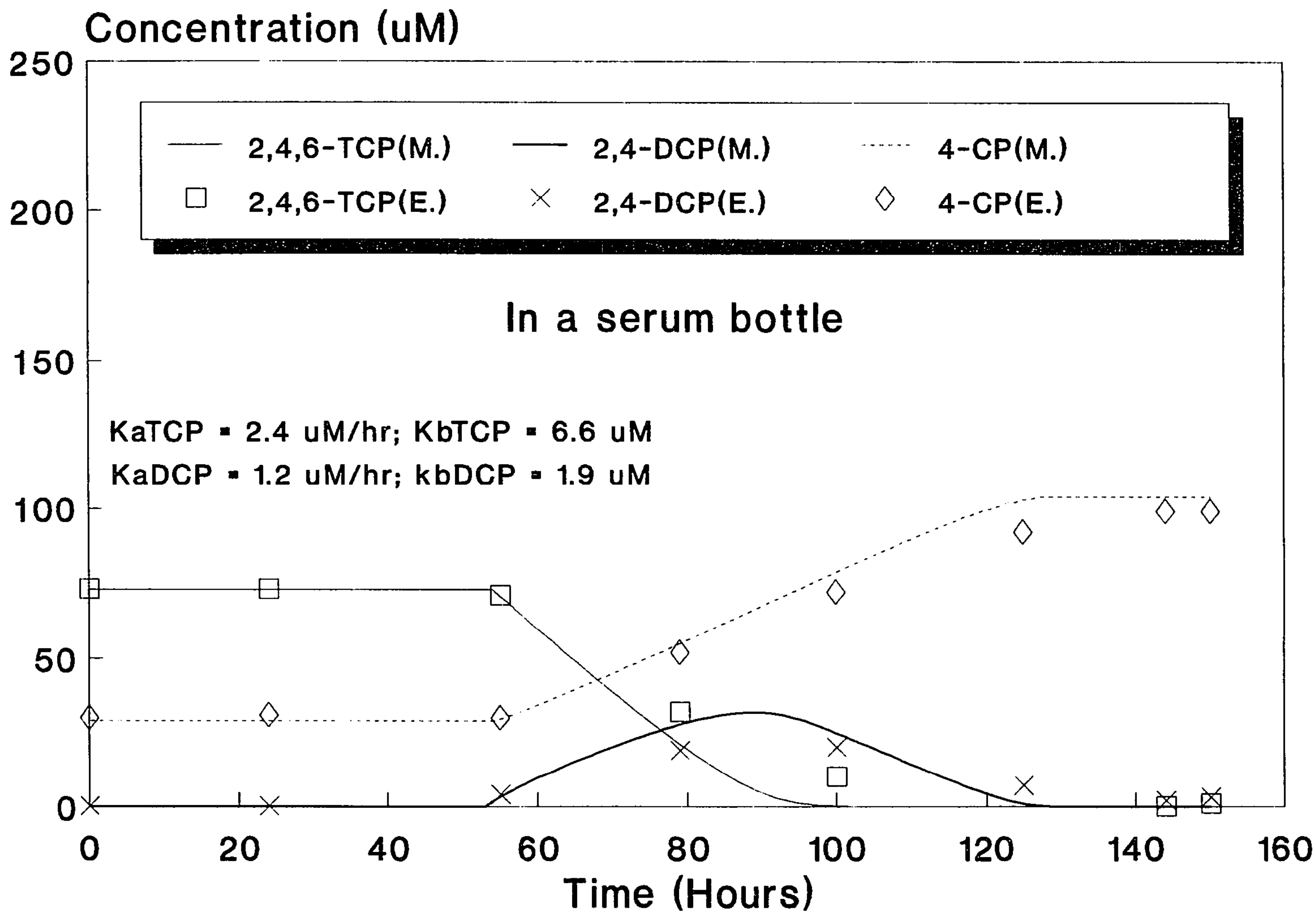

Figure 6-9 Experimental data and partially fitted curves for the anaerobic dahalogenation of 2,4,6-TCP (No. 2)

Table 6-4 Concentrations of 2,4,6-TCP 2,4-DCP, and 4-CP for anaerobic dehalogenation in a serum bottle

Time (hours)	2,4,6-TCP (μM) Expt. Output	2,4-DCP (μM) Expt. Output	4-CP (μM) Expt. Output	sum of CPs (μM) Expt.	CP % Reco.
0	73	0	30	103	100
24	73	0	31	104	101
55	71	4	30	105	102
79	32	19	52	101	98
100	10	20	72	102	99
125	0	7	92	99	96
144	0	2	99	101	98

* **A 125 ml serum bottle was used.**
The temperature was 30 ^{o}C

Table 6-5 (a), (b) and (c) Lists of kinetic constants for anaerobic dehalogenation of 2,4,6-TCP and 2,4-DCP with the methods of fitted curves, partially fitted curves, or predicted curves

Figure 6-5 (a)

Anaerobic Dehalogenation of 2,4,6-TCP (Data from Table 6-4)					
Kinetic Constants	K1a (μM/hr)	K1b (μM)	K2a (μM/hr)	K2b (μM)	Figure #
Fitted Curves Method	1.9	7.1	1.3	4.1	/
Partially Fitted Curves Method	2.4	6.6	1.2	1.9	Figure 6-9

Figure 6-5 (b)

Anaerobic Dehalogenation of 2,4,6-TCP (Data from Table 6-6)					
Kinetic Constants	K1a (μM/hr)	K1b (μM)	K2a (μM/hr)	K2b (μM)	Figure #
Fitted Curves Method	2.1	6.2	1.3	2.8	/
Predicted Curves Method	2.4	6.6	1.2	1.9	Figure 6-10

Figure 6-5 (c)

Anaerobic Dehalogenation of 2,4-DCP (Data from Table 6-8)			
Kinetic Constants	K2a (μM/hr)	K2b (μM)	Figure #
Fitted Curves Method	0.95	2.1	Figure 6-13
Partially fitted Curves Method	1.0	1.9	Figure 6-12

It is significant to notice that the ratios of the corresponding Ka values found from the regressions of the data shown in Figures 6-8 and 6-9 are all constant, i. e.:

$$\frac{Ka_{TCP} \text{ (Figure 6-8)}}{Ka_{TCP} \text{ (Figure 6-9)}} = \frac{3.2}{2.4} = \frac{Ka_{TCP} \text{ (Figure 6-8)}}{Ka_{TCP} \text{ (Figure 6-9)}} = \frac{1.6}{1.2}$$

This indicates that the biomass effect is indeed incorporated in the apparent Michaelis-Menten parameter Ka.

The values for the anaerobic dehalogenating Michaelis-Menten constants, Ka_{TCP}, Kb_{TCP}, Ka_{DCP}, and Kb_{DCP}, obtained the batch experiments of Figure 6-9 were then used to predict the behavior of the immobilized recirculating batch reactor. These values of Ka_{TCP} and Ka_{DCP} were somewhat better predictors than those of Figure 6-8, which was an earlier experiment. Table 6-6 gives the experimental data, and Figure 6-10 shows a comparison between the predicted curves (containing no fitted parameters), and the experimental data. The goodness of the agreement implies that the biomass concentration in the reactor was very similar to that in the serum bottle.

Finally, Figure 6-11 shows a comparison between the results of the prediction obtained using Equations 4, 5, and 6, and the experimental results from Table 6-7, for the anaerobic dehalogenation of 2,4,6-TCP in the immobilized recirculating continuous reactor. In this figure the operating parameter is the flow rate. The rate of anaerobic dehalogenation can be controlled via residence time (which is inversely proportional to the flow rate), and the results are shown in Figure 6-11 and Table 6-7. As the residence time decreased in the reactor, an increase in 2,4,6-TCP concentration was observed. As less 2,4,6-TCP is degraded, less 4-CP is formed. The fit between prediction

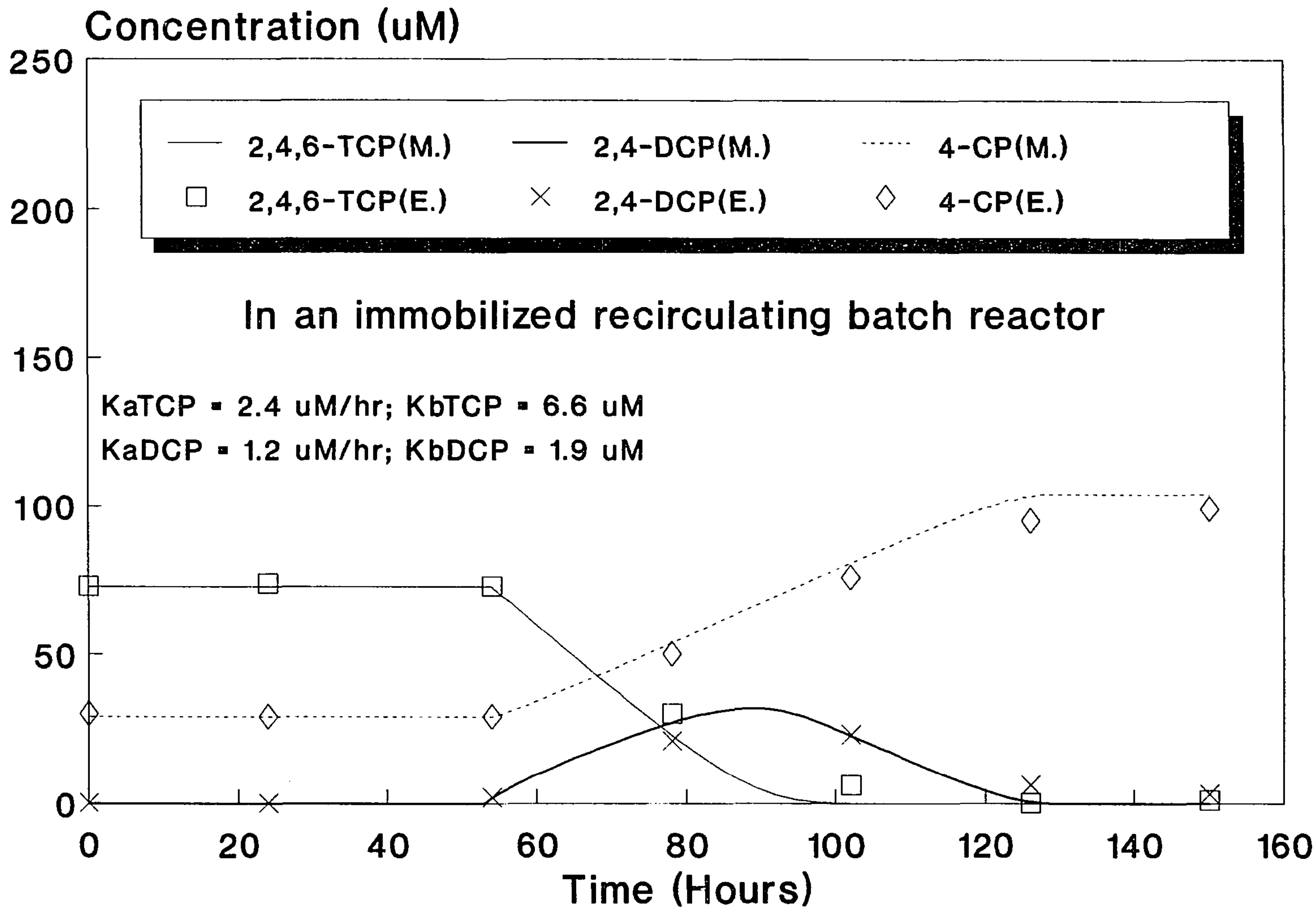

Figure 6-10 Experimental data and predicted curves for the anaerobic dehalogenation of 2,4,6-TCP

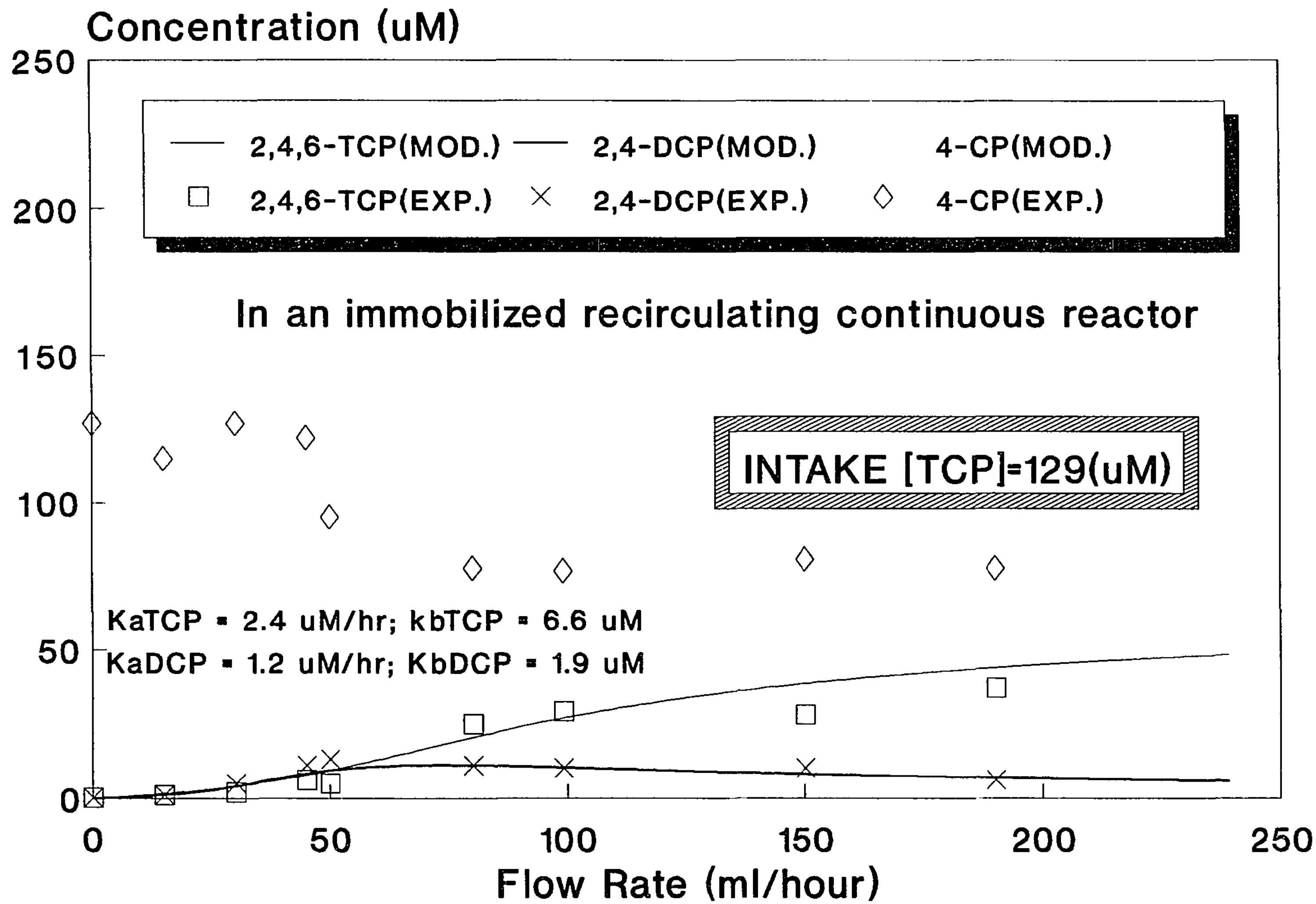

Figure 6-11 Experimental data and predicted curves for the continuous anaerobic dehalogenation of 2,4,6-TCP

Table 6-6 Concentrations of 2,4,6-TCP, 2,4-DCP, and 4-CP during the dehalogenation of 2,4,6-TCP in an anaerobic immobilized recirculating continuous reactor

Time (hours)	2,4,6-TCP (μM) Expt.	2,4-DCP (μM) Expt.	4-CP (μM) Expt.	sum of CPs (μM) Expt.	CP % Reco.	biomass (mg/100ml) Expt.
0	73	0	30	103	100	30
24	74	0	29	103	100	33
54	73	2	29	102	99	32
78	30	21	50	101	98	33.5
102	6	23	76	105	102	35
126	0	6	95	104	101	35
150	0	3	99	102	99	33

* **Anaerobic reactor volume: 5,300 (ml) & actual liquid volume: 2,000 (ml), pH: 8.1, and temperature at 30 ^{o}C**

Table 6-7 Concentrations of 2,4,6-TCP, 2,4-DCP, and 4-CP during the dehalogenation of 2,4,6-TCP using an anaerobic immobilized recirculating continuous reactor, at an intake concentration of 2,4,6-TCP = 129 μM

Flow Rate (ml/hr)	2,4,6-TCP (μM) Expt. Output	2,4-DCP (μM) Expt. Output	4-CP (μM) Expt. Output	sum of CPs (μM)	CP % Reco.
0	0	0	129	129	100
15	1	1	115	117	91
30	2	5	127	134	104
45	6	11	122	139	108
80	25	11	78	114	89
99	29	10	77	116	91
150	28	10	81	119	92
190	37	6	78	121	94

*** Anaerobic reactor volume: 5,300 (ml)**
Actual liquid volume: 2,000 (ml)
at 30 ^{o}C and pH: 8.1

and experimental results in Figure 6-11 is quite good. This indicates that the kinetic constants obtained from the serum bottle batch experiments are reliable indicators of the true kinetics of the system.

6.1.4 Results for the 2,4-DCP Dehalogenation Experiments and Their Interpretation Using the Proposed Model

In order to further test the anaerobic dehalogenation model for 2,4,6-TCP proposed above, additional serum bottle experiments were performed in which the same mixed culture was used to degrade 2,4-DCP alone. The experimental data are given in Table 6-8, and the modeling results are shown in Figures 6-12 and 6-13. Figure 6-12 was obtained using a value for Kb_{DCP} equal to 1.9 μM, (which was found previously for the anaerobic dehalogenation of 2,4,6-TCP), and by varying Ka_{DCP} until achieving a minimum in the sum of the absolute error. Figure 6-13 was obtained by fitting Equation 10 to the data and determining the best-fit values of Ka_{DCP} and Kb_{DCP}. From Figure 6-12 Ka_{DCP} was found to be 1.0 μM/hr, and from Figure 6-13 Ka_{DCP} and Kb_{DCP} were found to be 0.95 μM/hr and 2.1 μM, respectively. For both cases these kinetic constants are very close to the batch results found previously for 2,4,6-TCP (Ka_{DCP} = 1.2 μM/hr and Kb_{DCP} = 1.9 μM).

Figure 6-14 shows a similar experiment with another serum bottle containing a different amount of inoculum. The experimental data were taken from Table 6-9. The curves shown in this figure were obtained by maintaining the value (1.9 μM) of Kb_{DCP} obtained previously (since it is not a function of biomass). Ka_{DCP} was then varied until achieving the best fit, which was found to be 1.4 μM/hr. This value for Ka_{DCP} is again similar to the value of 1.2 μM/hr obtained from Figure 6-8.

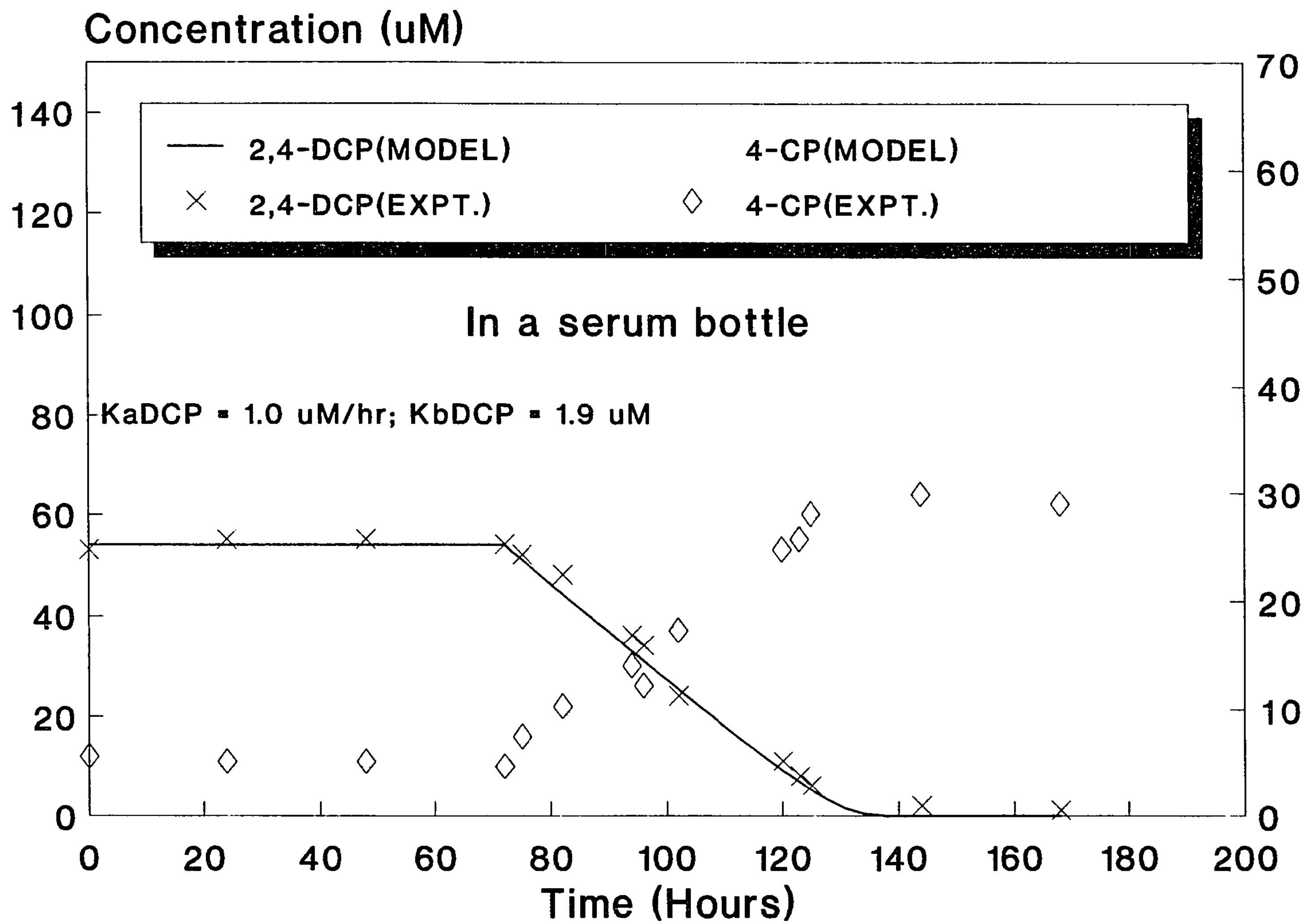

Figure 6-12 Experimental data and partially fitted curves for the anaerobic dehalogenation of 2,4-DCP (No. 1)

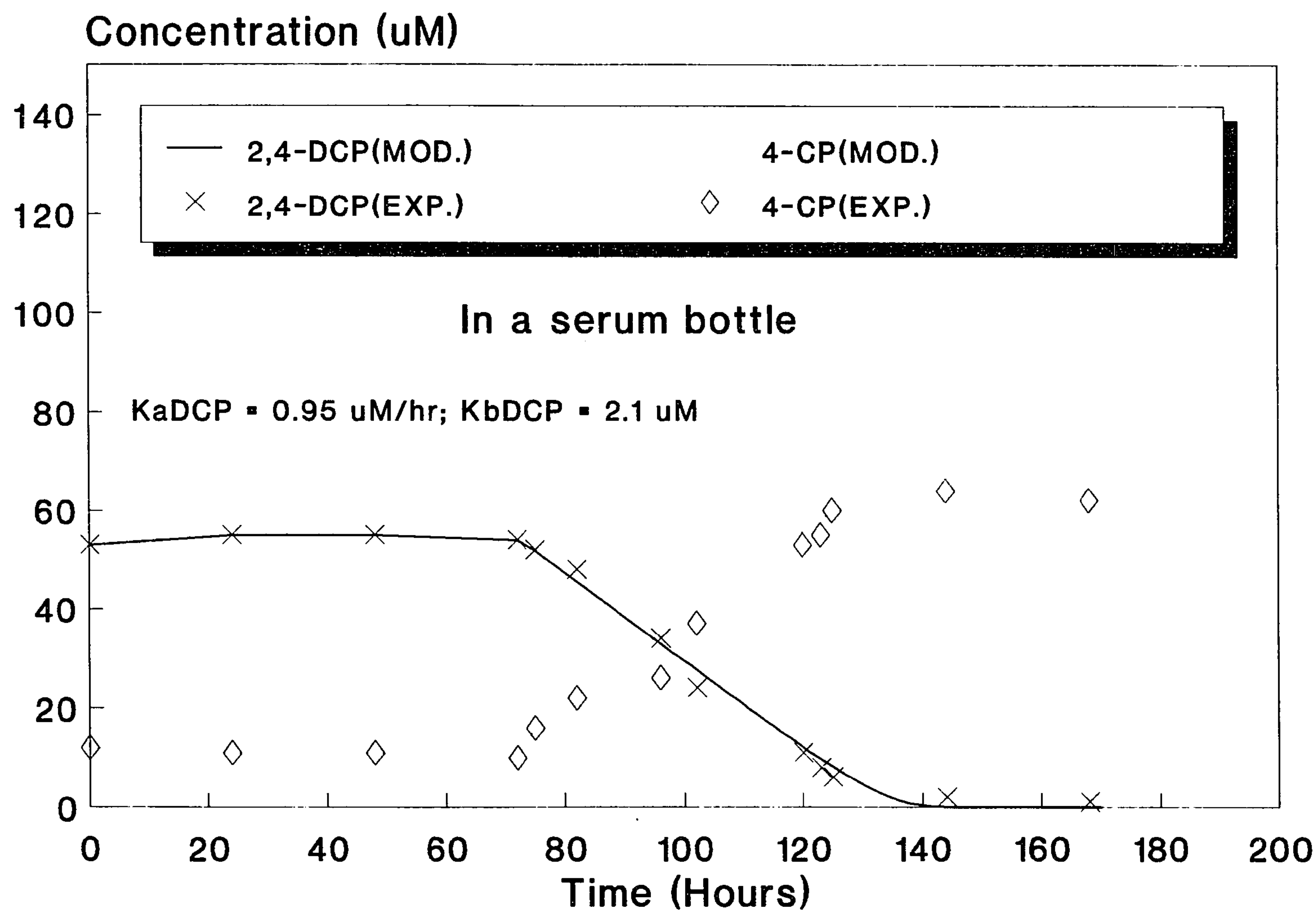

Figure 6-13 Experimental data and fitted curves for the anaerobic dehalogenation of 2,4-DCP (No. 1)

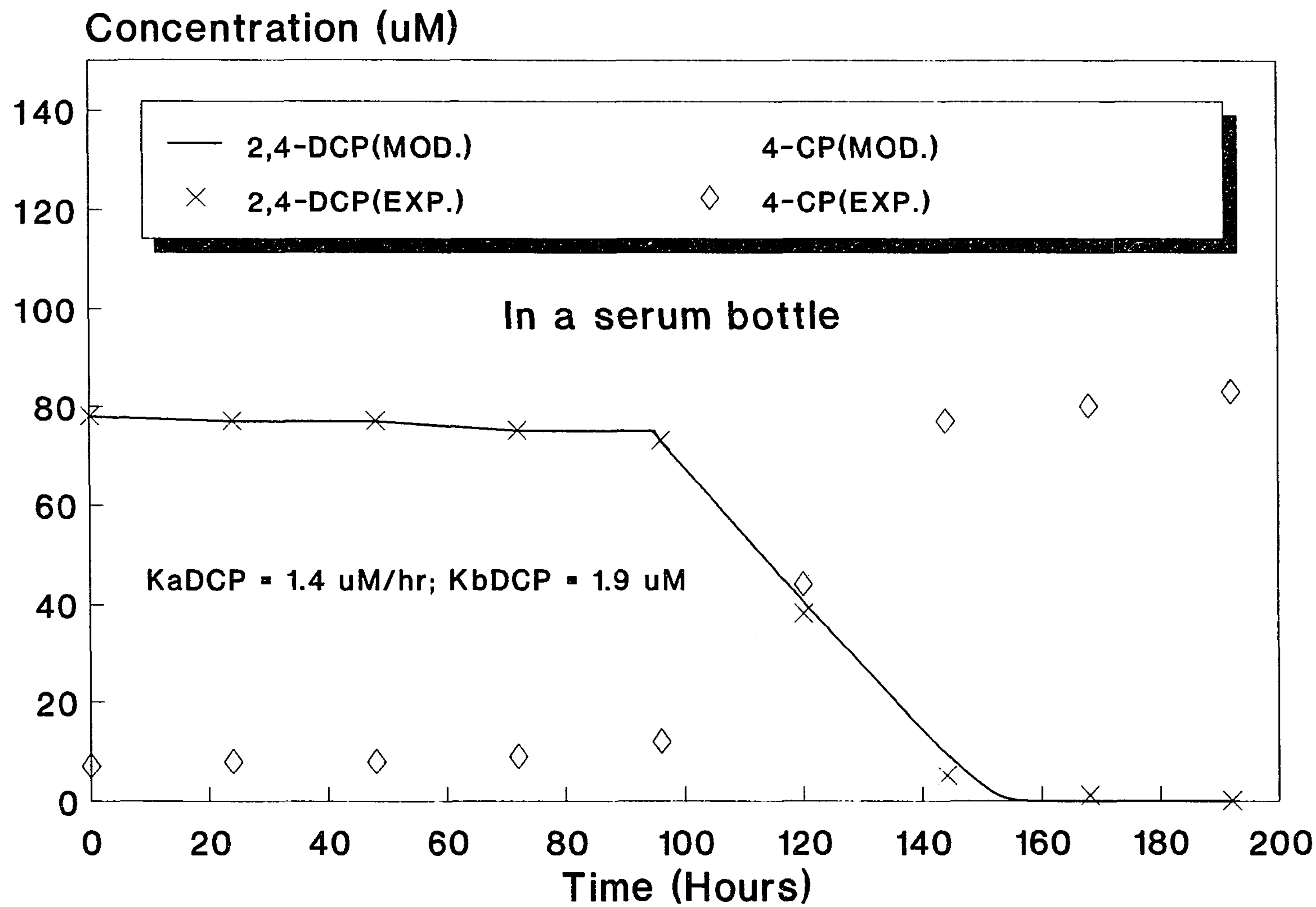

Figure 6-14 Experimental data and partially fitted curves for the anaerobic dehalogenation of 2,4-DCP (No. 2)

Table 6-8 Concentrations of 2,4-DCP and 4-CP during the anaerobic dehalogenation of 2,4-DCP in a shaker flask

Time (hours)	pH	2,4-DCP (μM) Expt.	4-CP (μM) Expt.	sum of CPs (μM)	CP % Reco.
0	8.1	53	12	65	100
24	/	55	11	66	102
48	/	55	11	66	102
72	/	54	10	64	98
75	/	52	16	68	105
82	/	48	22	70	108
96	/	34	26	60	92
102	/	24	37	61	94
120	/	11	53	64	98
123	/	8	55	63	97
125	/	6	60	66	102
144	8.2	2	64	66	102
168	8.2	1	62	63	97

* A 125 ml serum bottle was used.
The temperature was 30 ^{o}C

Table 6-9 Concentrations of 2,4-DCP and 4-CP during the anaerobic dehalogenation of 2,4-DCP over a period of 10 days

Time (days)	2,4,6-TCP (µM)	2,4-DCP (µM)	4-CP (µM)	sum of CPs (µM)	CP % Reco.
0	0	78	7	85	100
1	0	77	8	85	100
2	0	77	8	85	100
3	0	75	9	84	99
4	0	73	12	85	100
5	0	38	44	82	96
6	0	5	77	82	96
7	0	1	80	81	95
8	0	0	76	76	89
9	0	0	79	79	93
10	0	0	78	78	92

* A 125 ml serum bottle was used.
The temperature was 30 ^{o}C

6.1.5 The Effect of 2,4,6-TCP and 4-CP Concentration on 2,4,6-TCP Anaerobic Dehalogenation

This experiment was designed to determine if dehalogenation would also take place at high concentrations of 2,4,6-TCP. When the concentration of 2,4,6-TCP was between 40 and 150 μM, no inhibition or toxicity effects were observed. However the reaction was totally inhibited when the concentration of 2,4,6-TCP was 908 μM (Figure 6-15). Increasing the concentration of 4-CP to 923 μM also led to inhibition (Figure 6-16).

6.1.6 The Effect of Temperature on 2,4,6-TCP Anaerobic Dehalogenation

The dehalogenation of 2,4,6-TCP was performed in anaerobic serum bottles at 19 ^{o}C, 25 ^{o}C and 30 ^{o}C. At 19 ^{o}C (Figure 6-17a and Table 6-10), the degradation did not begin until approximately 70 hours into the experiment. Slow degradation of 2,4,6-TCP was observed. The experiment at 25 ^{o}C (Figure 6-17b and Table 6-11) further confirmed the first observation. However, when the temperature was raised to 30 ^{o}C the rate of dehalogenation increased as observed in Figure 6-17c and Table 6-12.

In all three experiments, there was the initial lag time before the degradation began. The lag time observed in each case decreased as the temperature was raised.

Table 6-10, 6-11, and 6-12 show the experimental data for anaerobic dehalogenation as a function of temperature over a range of 19 - 30 ^{o}C. These data are interpreted assuming an Arrhenius type equation for both Ka_{TCP} and Ka_{DCP}, i.e., using Equations 5.14 and 5.15 given in the previous chapter. It was assumed that Kb_{TCP} and Kb_{DCP} are not functions of temperature. Ka_{TCP} and Ka_{DCP} were obtained through regression of the experimental data. Figures 6-18, 6-19, and 6-20 show the resulting values of Ka_{TCP} and Ka_{DCP} at

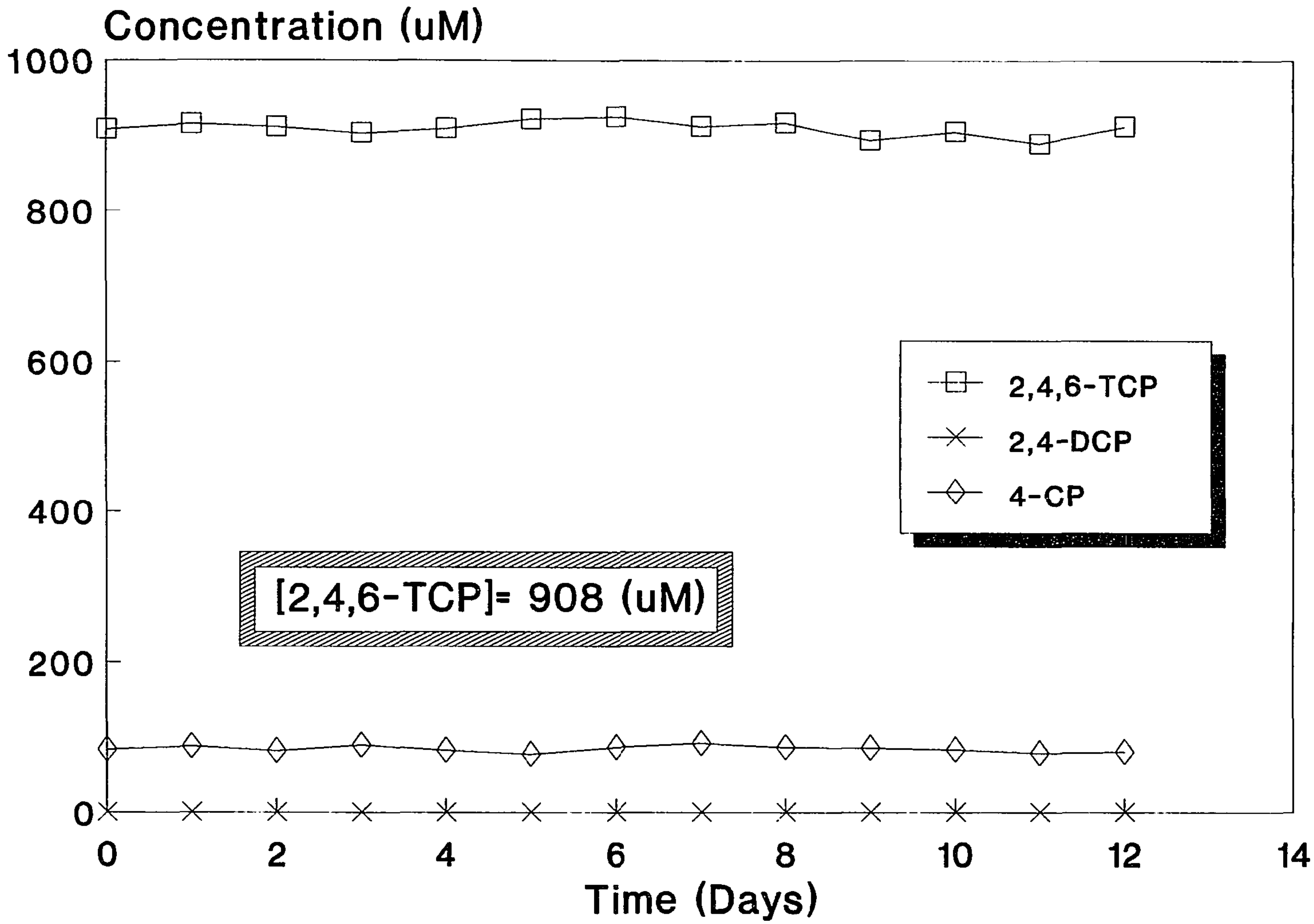

Figure 6-15 Effect of high concentration of 2,4,6-TCP on the anaerobic dehalogenation of 2,4,6-TCP

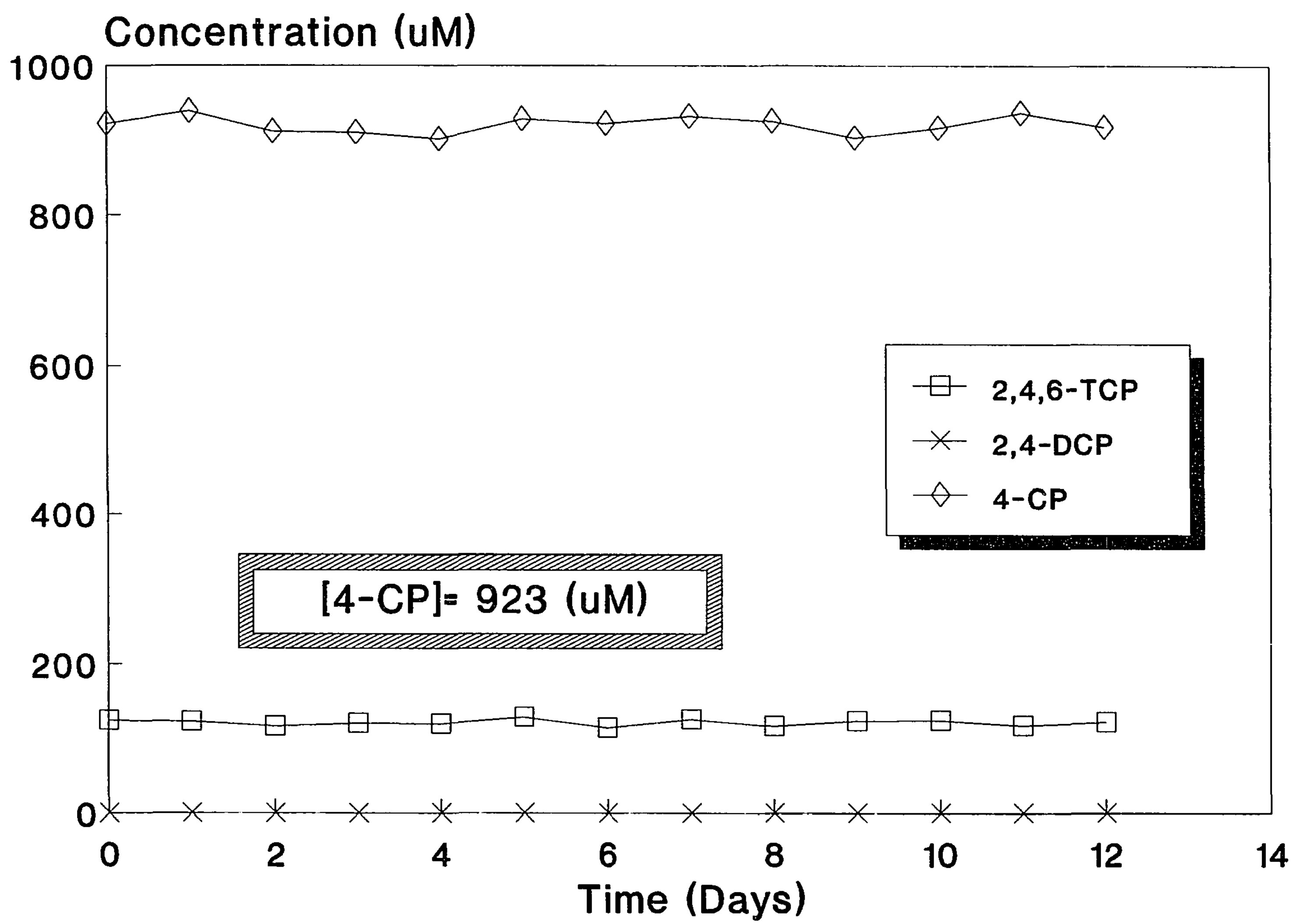

Figure 6-16 Effect of high conctentration of 4-CP on the anaerobic dehalogeantion of 2,4,6-TCP

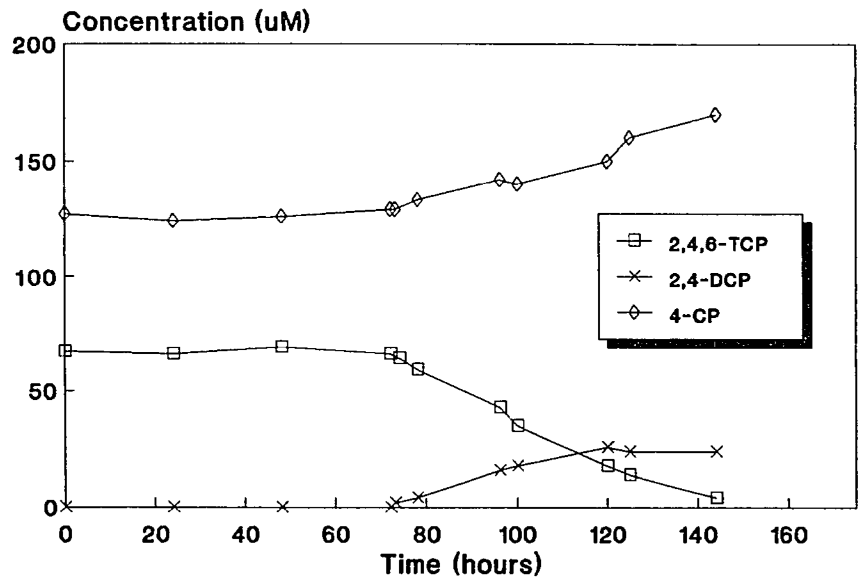

Figure 6-17(a) Dehalogenation of
2,4,6-TCP at 19 oC

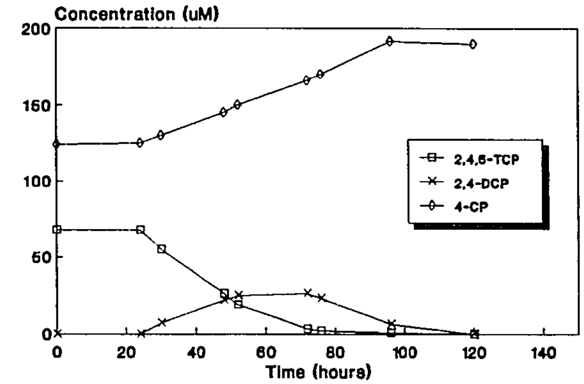

Figure 6-17(b) Dehalogeantion of
2,4,6-TCP at 25 oC

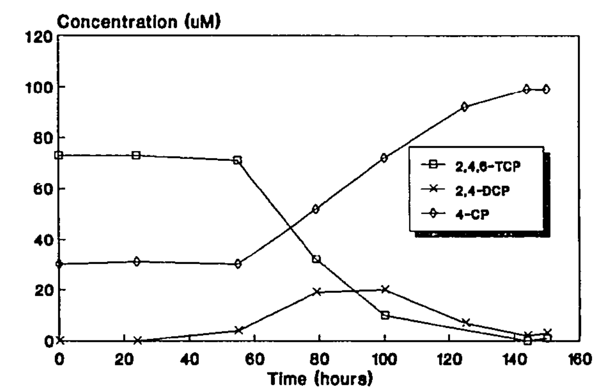

Figure 6-17(c) Dehalogenation of
2,4,6-TCP at 30 oC

Figure 6-17 Effect of temperature on the anaerobic dehalogeantion of 2,4,6-TCP at 19 oC, 25 oC, and 30 oC

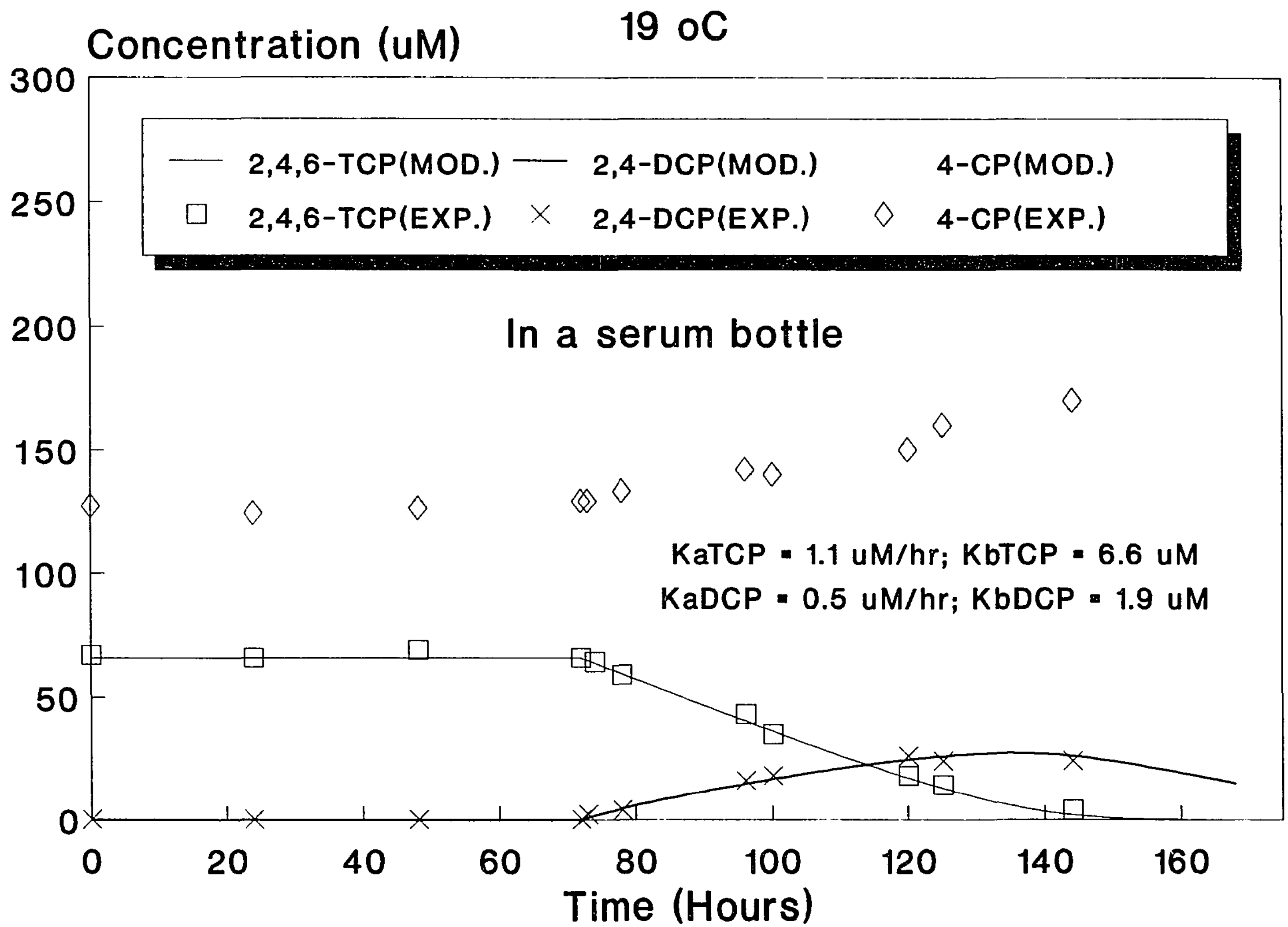

Figure 6-18 Experimental data and partially fitted curves for the effect of temperature on 2,4,6-TCP dehalogenation

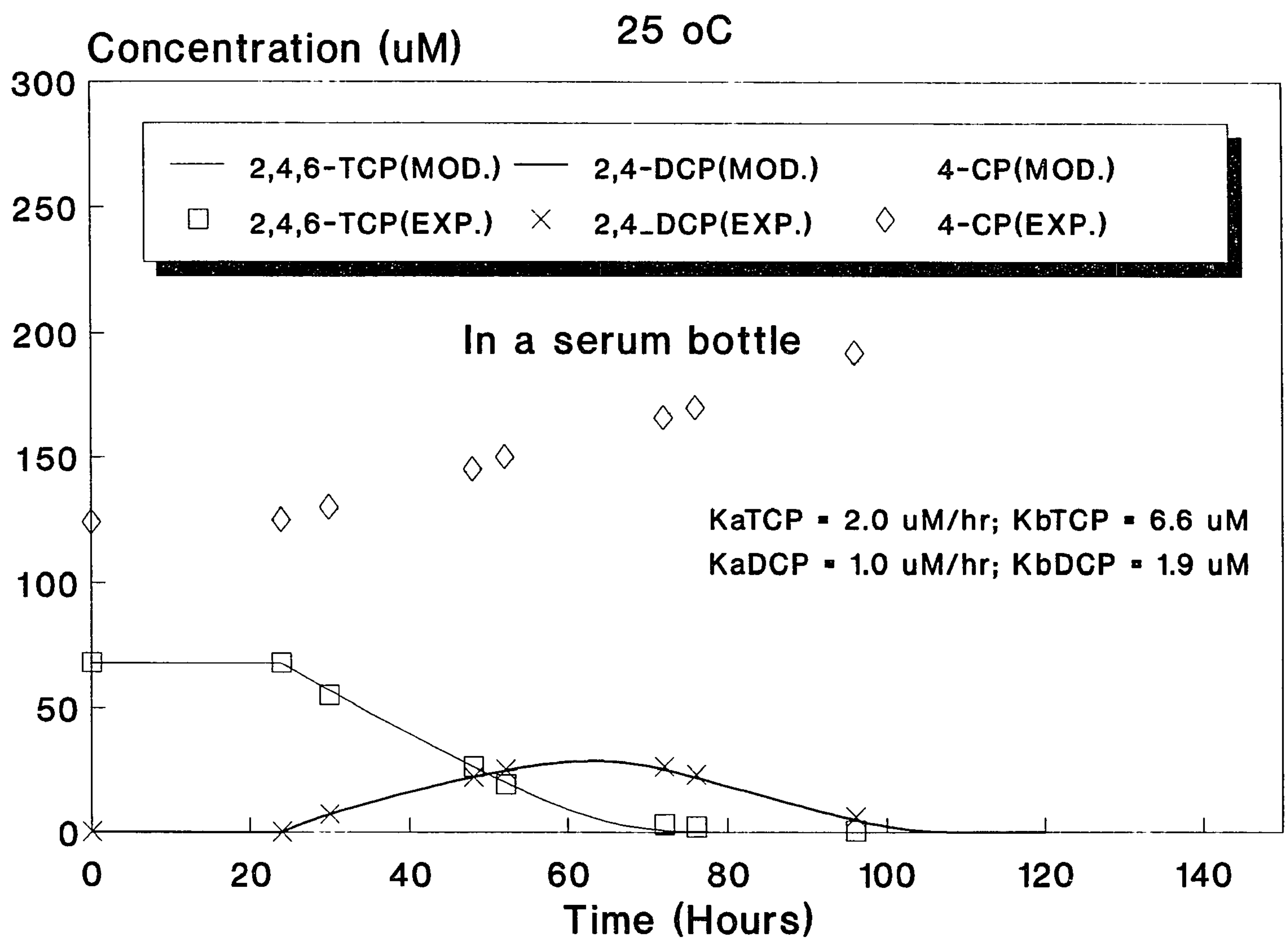

Figure 6-19 Experimental data and partially fitted curves for the effect of temperature on 2,4,6-TCP dehalogenation

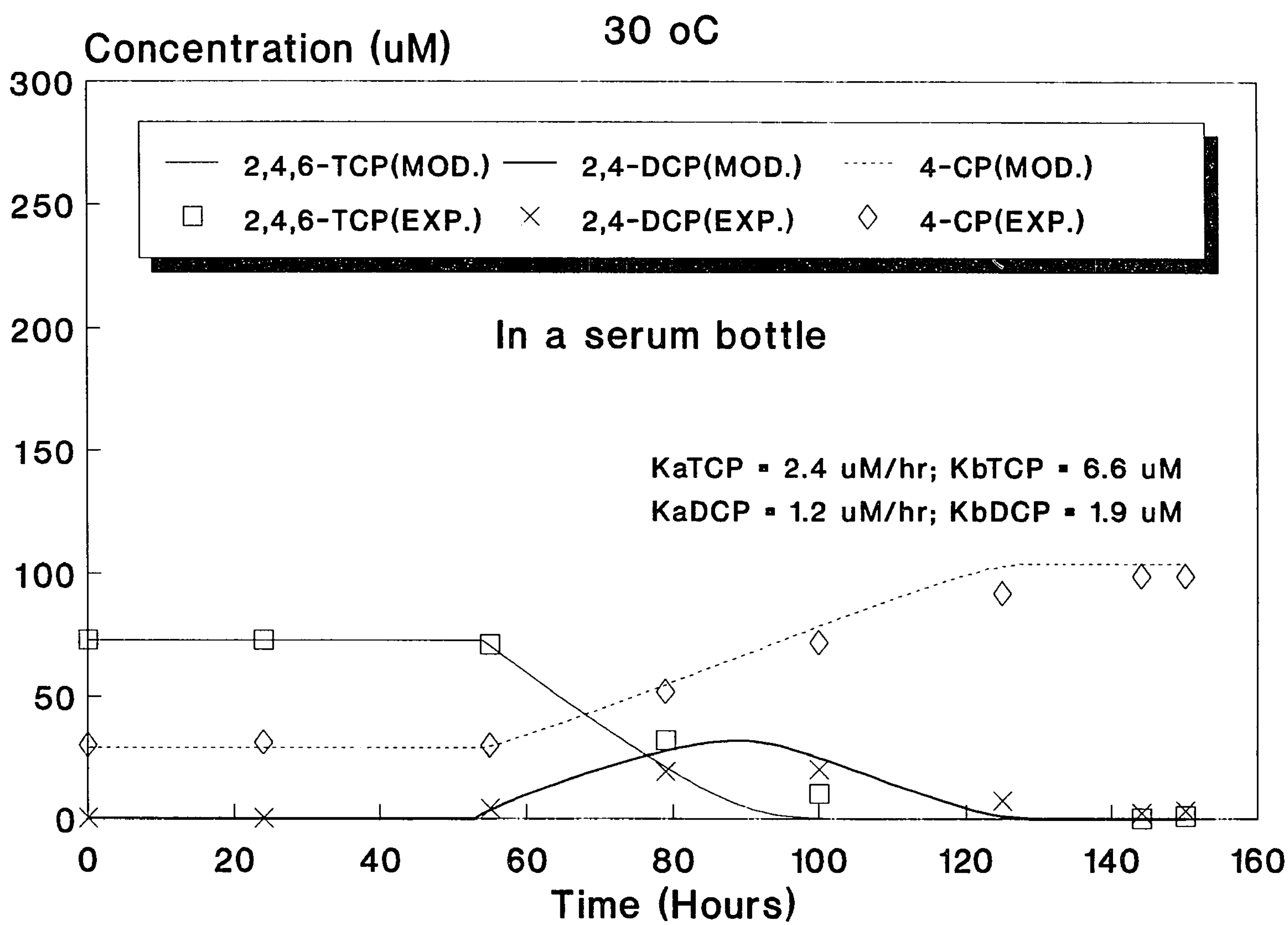

Figure 6-20 Experimental data and partially fitted curves for the effect of temperature on 2,4,6-TCP dehalogenation

Table 6-10 The effect of temperature on anaerobic dehalogenation of 2,4,6-TCP in a serum bottle at T = 19 ^{o}C

Time (hours)	2,4,6-TCP (μM) Expt. Output	2,4-DCP (μM) Expt. Output	4-CP (μM) Expt. Output	sum of CPs (μM) Expt.	CP % Reco.
0	67	0	129	196	100
24	66	0	129	195	99
48	69	0	130	199	102
72	66	0	129	195	99
74	64	2	129	194	99
78	59	4	133	196	100
96	43	16	142	201	103
100	35	18	141	194	99
120	18	26	150	194	99
125	14	24	160	198	101
144	4	24	171	199	102

* A 125 ml serum bottle was used.
The temperature was 19 ^{o}C

Table 6-11 The effect of temperature on anaerobic dehalogenation of 2,4,6-TCP in a serum bottle at T = 25 ^{o}C

Time (hours)	2,4,6-TCP (µM) Expt. Output	2,4-DCP (µM) Expt. Output	4-CP (µM) Expt. Output	sum of CPs (µM) Expt.	CP % Reco.
0	68	0	124	192	100
24	68	0	125	193	101
25	66	3	125	194	101
30	55	7	130	192	100
48	26	22	145	193	101
52	19	25	150	194	101
72	3	26	166	195	102
76	2	23	170	195	102
96	1	6	192	199	104

* **A 125 ml serum bottle was used.**
The temperature was 25 ^{o}C

Table 6-12 The effect of temperature on anaerobic dehalogenation of 2,4,6-TCP in a serum bottle at T = 30 ^{O}C

Time (hours)	2,4,6-TCP (μM) Expt. Output	2,4-DCP (μM) Expt. Output	4-CP (μM) Expt. Output	sum of CPs (μM) Expt.	CP % Reco.
0	73	0	30	103	100
25	73	0	31	104	101
56	72	4	31	105	102
78	32	19	52	101	98
100	9	21	72	102	99
125	0	7	94	101	98
144	0	0	101	101	98

*** A 125 ml serum bottle was used.**
The temperature was 30 ^{O}C

19, 25, and 30 ^oC, respectively. Ka_{TCP} and Ka_{DCP} were found to be equal to 1.1 and 1.1 μM/hr at 19 ^oC, 2.0 and 1.0 μM/hr at 25 ^oC, and 2.4 and 1.2 μM/hr at 30 ^oC. These results indicate that the temperature has a significant effect on the anaerobic dehalogenation kinetic constants, Ka_{TCP} and Ka_{DCP}.

Figure 6-21 and Table 6-13 presents the resulting value of E_{TCP} which is equal to 13,000 cal/mole, and Figure 6-22 and Table 6-14 reports the resulting value of E_{DCP} which is equal to 14,700 cal/mole. Metcalf and Eddy (1972) reported that the activation energy, Ea, for biological waste water treatment processes is generally within the range 2,000 to 20,000 cal/mole, which is in agreement with the values obtained in this work.

6.1.7 Experiments to Determine the Anaerobic Dehalogenation of PCP, 2,4-DCP, and 4-CP

Figure 6-23 and Table 6-15 shows that no PCP was anaerobically degraded for the first 10 days after inoculation. Furthermore, Figure 6-24 and Table 6-16 shows that no 4-CP was anaerobically degraded for the first 10 days after inoculation.

We concluded that 4-CP and PCP are not dechlorinated, or only very slowly dechlorinated, in the anaerobic process. In further investigation, 4-CP was found to be totally mineralized after 3 months. However, PCP was not significantly biodegraded even after 3 months.

Although no anaerobic dehalogenation of PCP or 4-CP occurred over a period of 10 days, when 68 μM of 2,4,6-TCP were added to both cultures, it was found that 2,4,6-TCP was dechlorinated to 4-CP in 3 days. This shows that the cultures did not lose their dehalogenating activity against 2,4,6-TCP, but that they were inactive against PCP and 4-CP.

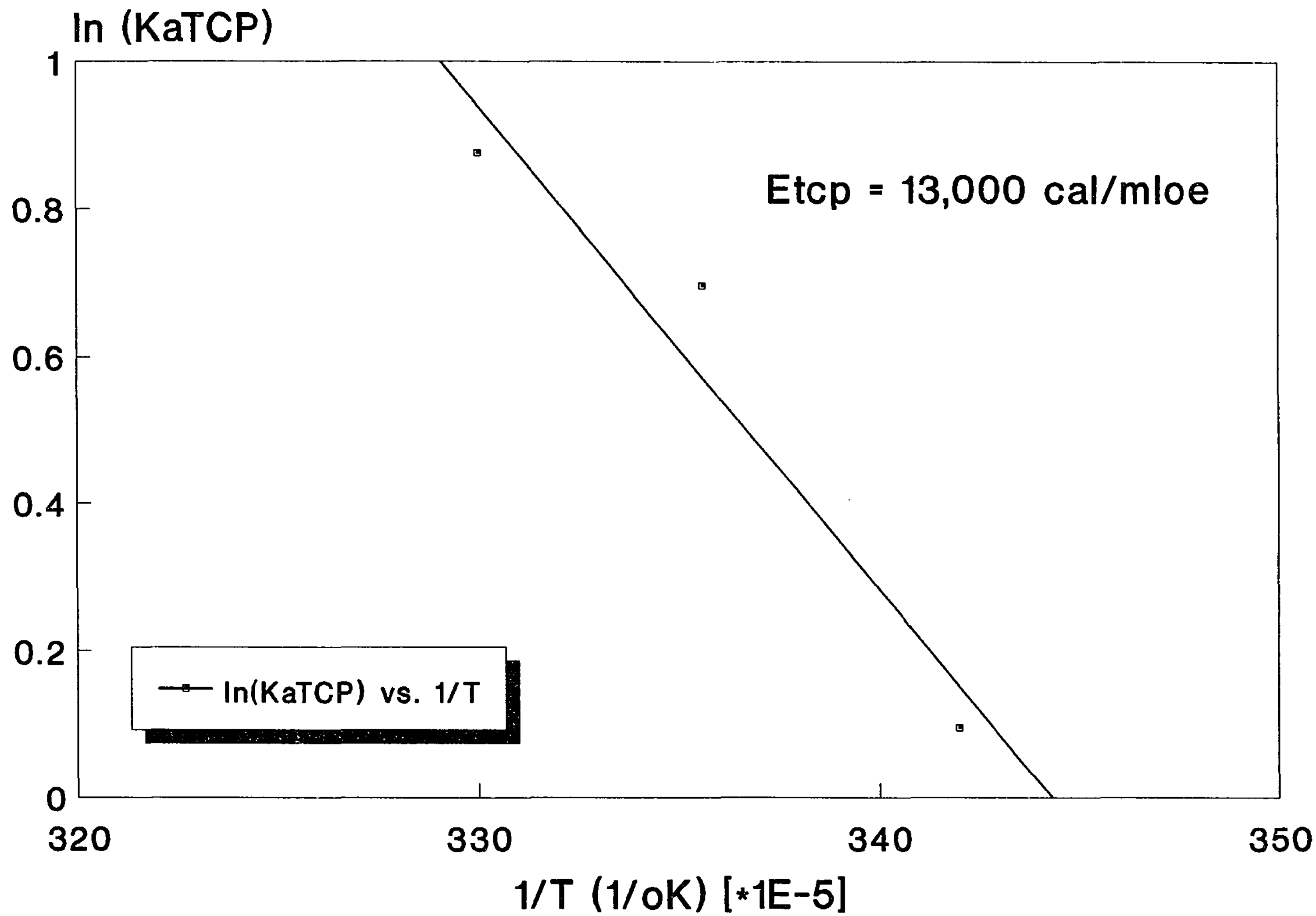

Figure 6-21 Arrhenius plot for KaTCP

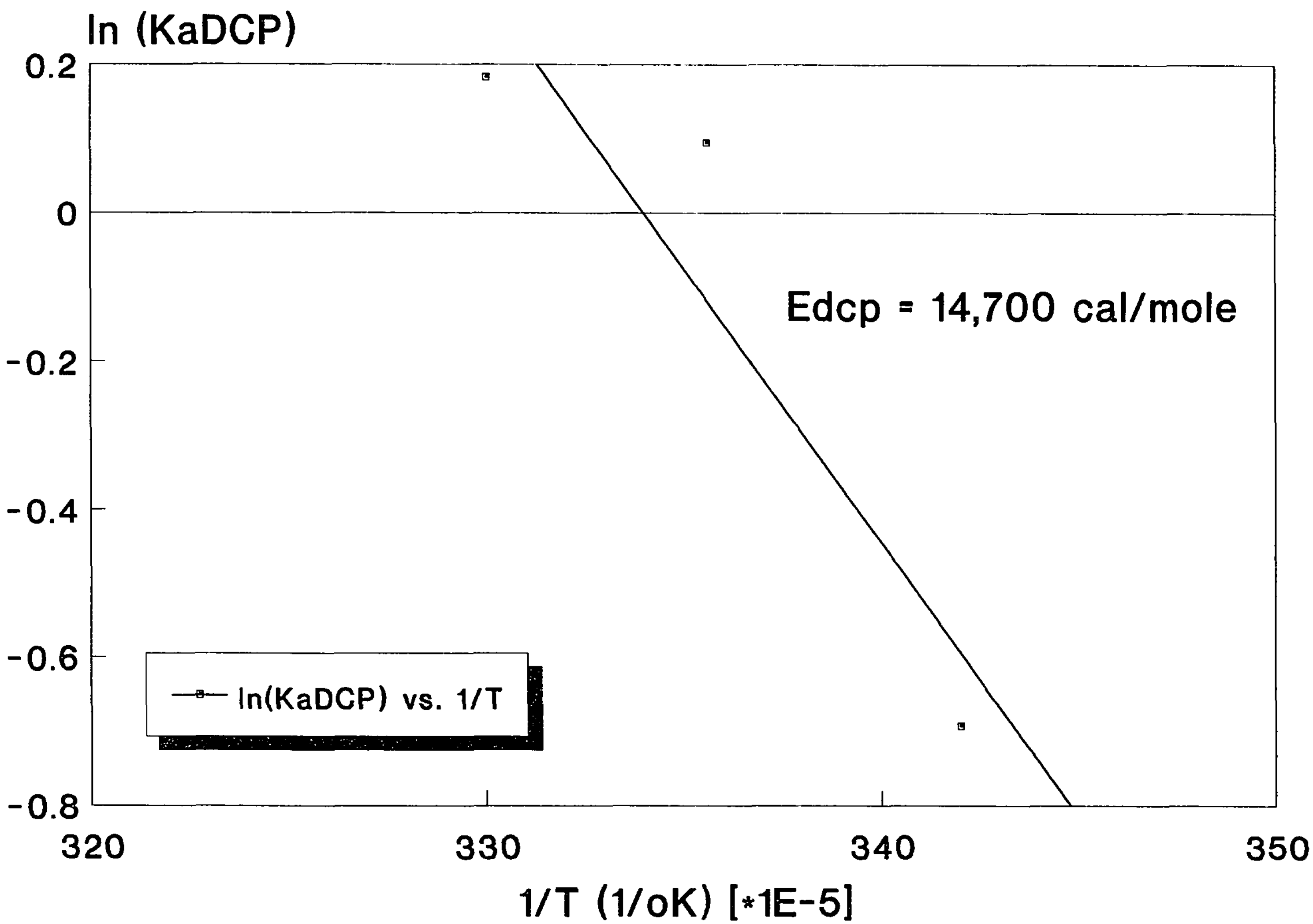

Figure 6-22 Arrhenius plot data for KaDCP

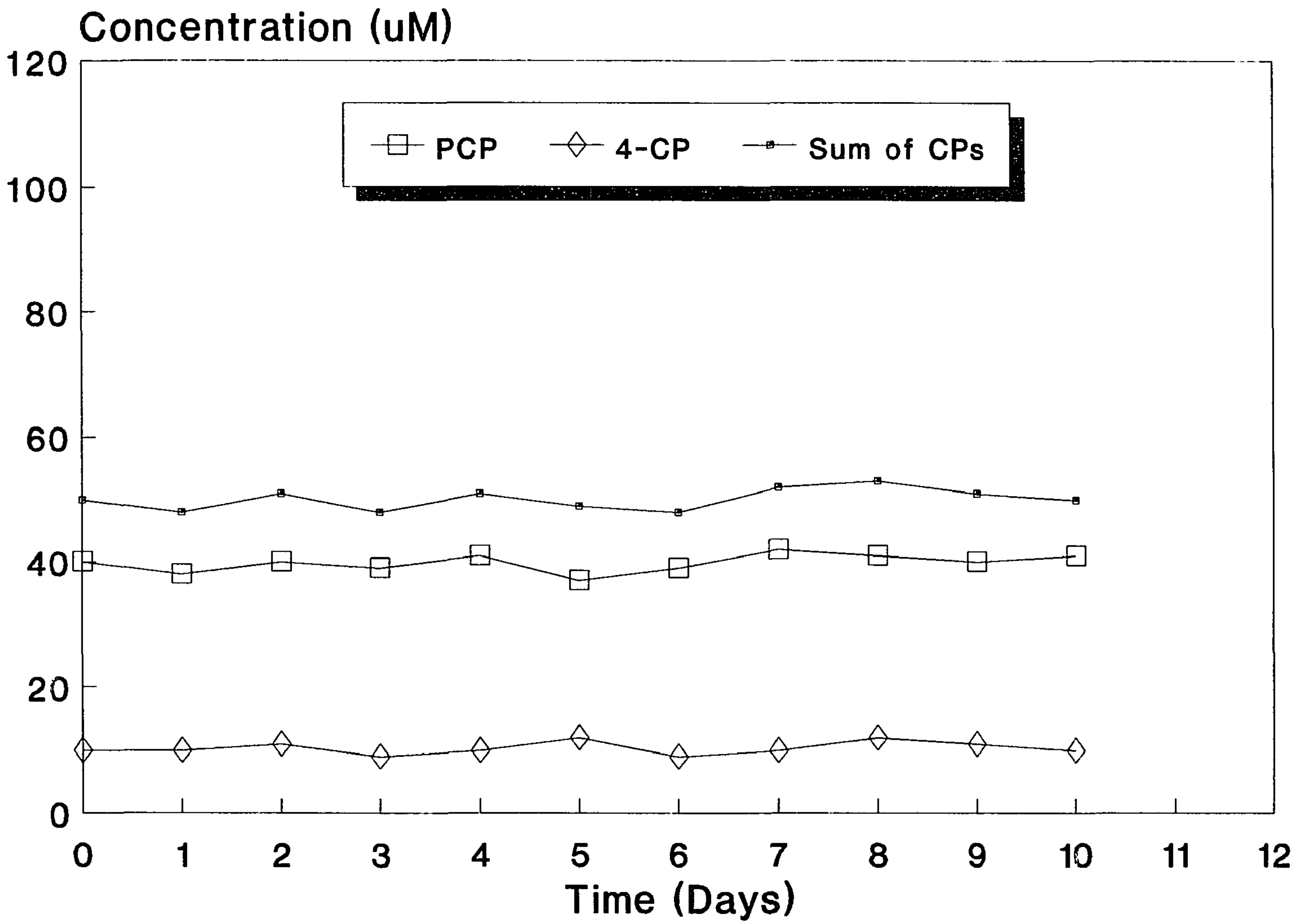

Figure 6-23 Results of experiments on the anaerobic dehalogenation of PCP

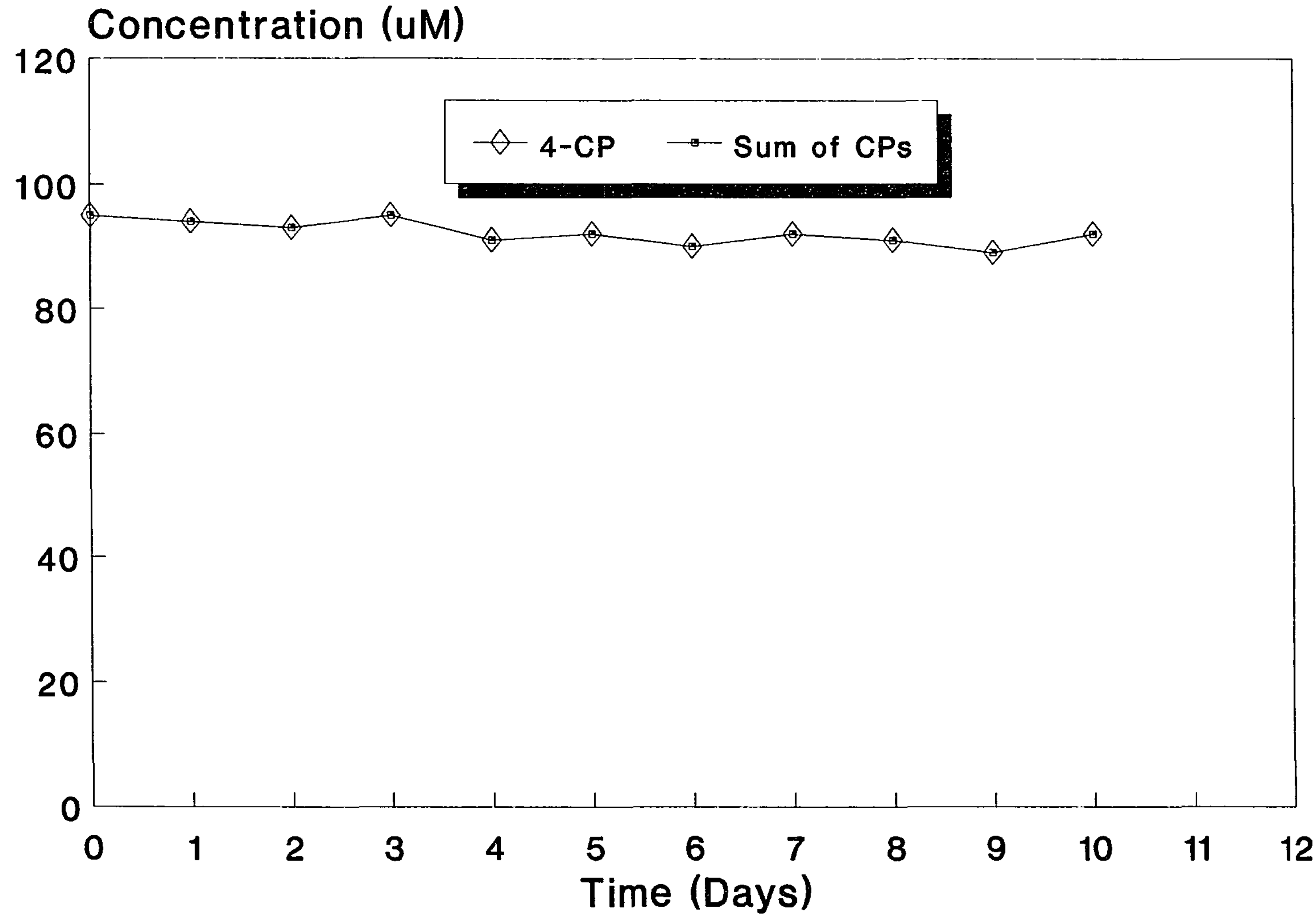

Figure 6-24 Results of experiments on the anaerobic dehalogenation of 4-CP

Table 6-13 Effect of temperature on Ka_{TCP} during the anaerobic dehalogenation of 2,4,6-TCP

T (°C)	T (°K)	1/T (°K)	Ka_{TCP} (µM/hr)	ln (Ka_{TCP})
30	303	0.033	2.4	0.8755
25	298	0.03356	2.0	0.6931
19	292	0.00342	1.1	0.0935

Table 6-14 Effect of temperature on Ka_{DCP} during the anaerobic dehalogenation of 2,4,6-TCP

T (°C)	T (°K)	1/T (°K)	Ka_{DCP} (µM/hr)	ln (Ka_{DCP})
30	303	0.033	1.2	0.1823
25	298	0.03356	1.0	0.0935
19	292	0.00342	0.5	- 0.6931

Table 6-15 Concentrations of PCP and 4-CP during the anaerobic dehalogenation of PCP over a period of 10 days

Time (days)	PCP (µM)	4-CP (µM)	sum of CPs (µM)	CP % Reco.
0	40	10	50	100
1	38	11	48	96
2	40	11	51	102
3	39	9	48	96
4	41	10	51	102
5	37	12	49	98
6	39	9	48	96
7	42	10	52	104
8	41	12	53	106
9	40	11	51	102
10	41	10	51	102

* A 125 ml serum bottle was used.
The temperature was 30 ^{o}C

Table 6-16 Concentration of 4-CP during the anaerobic dehalogenation of 4-CP over a period of 10 days

Time (days)	4-CP (μM)	sum of CPs (μM)	CP % Reco.
0	95	95	100
1	94	94	99
2	93	93	98
3	95	95	100
4	91	91	96
5	92	92	97
6	90	90	95
7	92	92	97
8	91	91	96
9	89	89	94
10	92	92	97

* **A 125 ml serum bottle was used.**
The temperature was 30 ^{o}C

6.2 Aerobic Process

6.2.1 Results of the Mineralization of 4-CP under Aerobic Conditions and Their Interpretation Using the Proposed Model

The results of the experiments in which the pH in the aerobic culture was changed showed that no mineralization of 4-CP occurred when the pH was maintained at 6.5, 7.8 or 9.5. However, mineralization took place when the medium was buffered at 7.2. Figure 6-25 shows the results of these experiments. Because of the small pH fluctuation observed in the experiments it can be concluded that the pH range in which aerobic mineralization occurred is 7.0 -7.5.

Similarly to what observed in the anaerobic system, aerobic mineralization of 4-CP occurred upon the establishment of the approximate pH even if the cultures had been previously exposed to different pH's at which no mineralization occurred. More specifically, cultures exposed to pH's equal to 6.5, 7.8 or 9.5 (at which no degradation was observed for as long as seven days) started mineralizing 4-CP when the pH was brought to 7.2. No lag time was observed once the pH was changed to the new value.

When the pH value was adjusted in the range 7.0 - 7.5, the degradation of 4-CP in the aerobic batch system was readily obtained, as shown in Figure 6-26 and Table 6-17. The sharp decrease in 4-CP concentration during the first 8 hours, from 66 μM to near complete mineralization, proves the effectiveness of the aerobic system in degrading mono-halo aromatic. A control experiment (Figure 6-27) showed no decrease in 4-CP concentration in an aerated reactor containing only sterile medium.

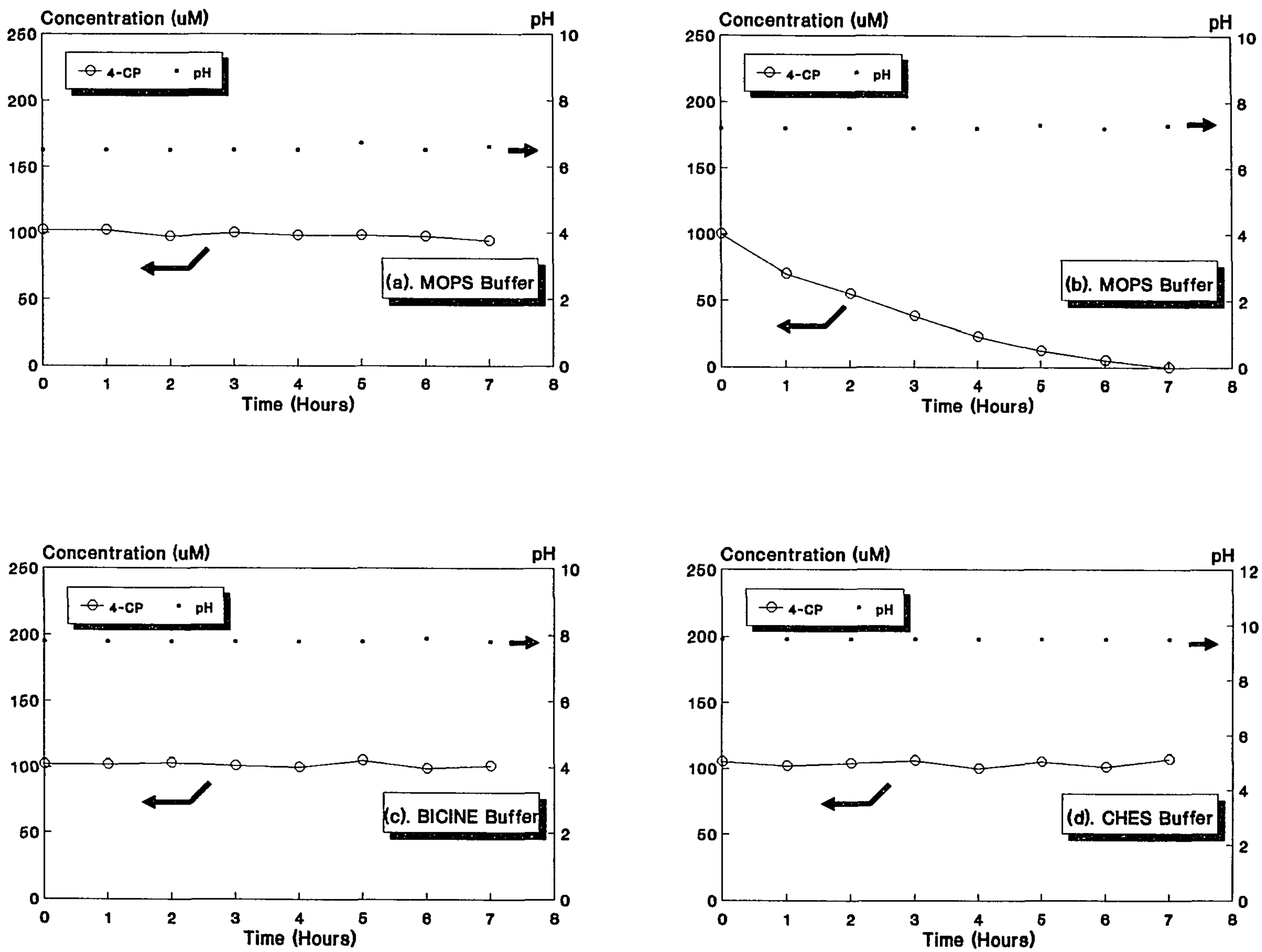

Figure 6-25 Effect of pH on mineralization of 4-CP

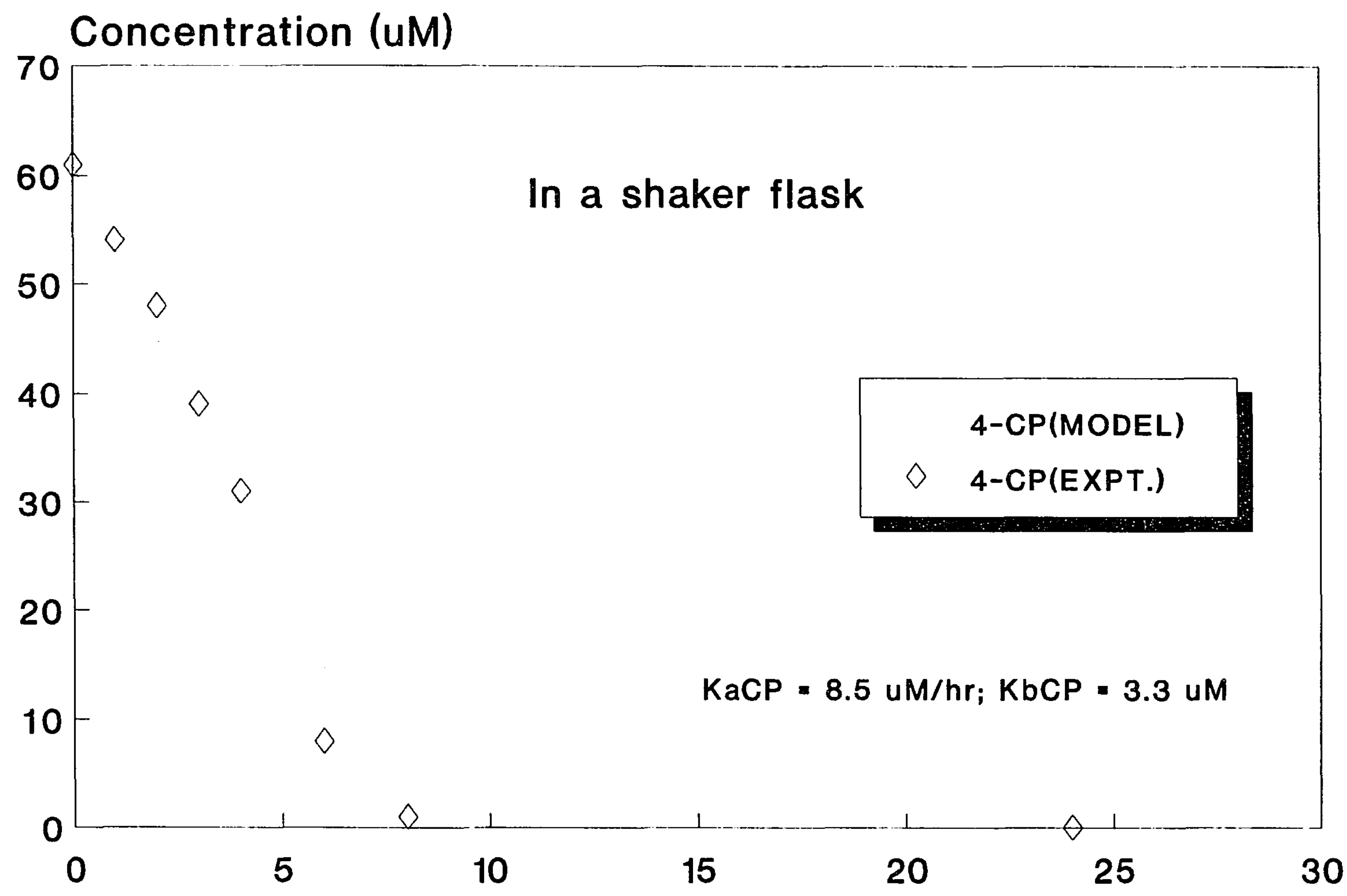

Figure 6-26 Experimental data and fitted curves for the aerobic mineralization of 4-CP

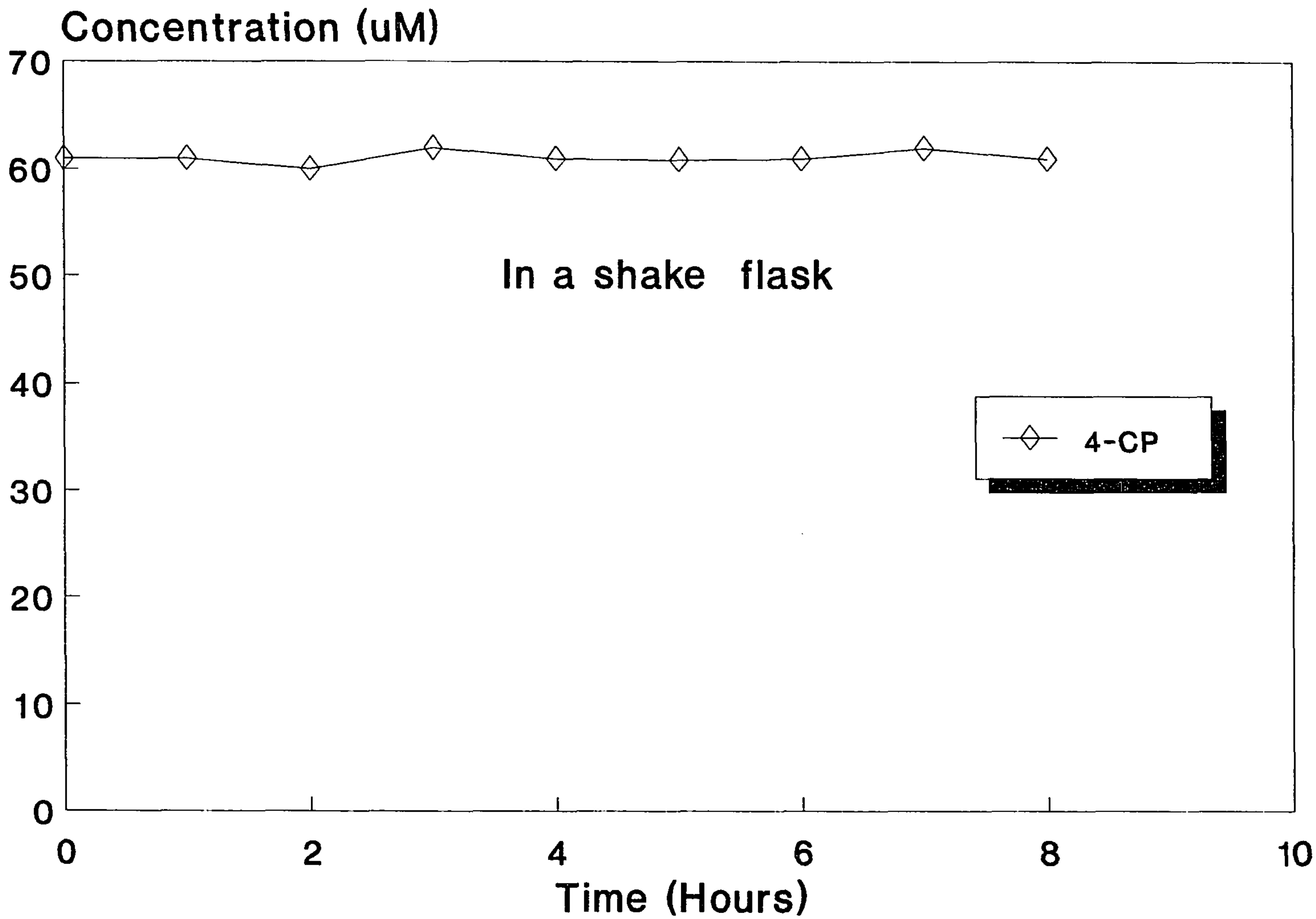

Figure 6-27 Control experiment for the aerobic mineralization of 4-CP in a shake flask

Table 6-17 Concentration of 4-CP during the aerobic mineralization of 4-CP in a shaker flask using Medium B with buffer MOPS

Time (hours)	pH	4-CP (µM)	sum of CPs (µM)	Cl (µM)	Cl^- differ. (µM)	Biomass (mg/ 100ml)
0	7.1	61	61	740	0	190
1	7.1	57	57	/	/	190
2	7.1	48	48	/	/	140
3	7.1	39	39	/	/	190
4	7.1	31	31	770	30	170
6	7.2	8	8	780	40	170
8	7.2	1	1	790	50	150
24	7.2	0	0	810	70	190

* **System:**

A 150 ml flask was placed in the New Brunswick Controlled Environment Incubator Shaker

The experimental 4-CP mineralization data were used to obtain Ka_{CP} and Kb_{CP} via linear regression of the integral of Equation 5.11. Ka_{cp} and Kb_{cp} were found to be 8.5 μM/hr and 3.3 μM, respectively, as shown in Figure 6-26.

Figure 6-28 shows the results for the aerobic suspended cell batch reactor. The data in this figure are from Table 6-18, and were fitted using the value of Kb_{CP} (= 3.3 μM) from the shaker flask experiment (i.e., Figure 6-27). Ka_{CP} was found to be 10 μM/hr, which is close to the value of Ka (= 8.5 μM/hr) obtained from Figure 6-26.

The Michaelis-Menten constants for the mineralization of 4-CP (obtained from the suspended cell batch reactor) were applied to predict the behavior of the suspended cell continuous reactor as a function of flow rate over the range 0 - 190 ml/hr. The experimental data are taken from Table 6-18. Figure 6-29 shows the comparison between the model prediction and the experimental results. The agreement is very good.

Again, as expected, as the residence time decreased, less 4-CP was biodegraded (as shown in Figure 6-29 and Table 6-19).

6.2.2 Aerobic Degradation of 3-CP

The effectiveness of aerobic degradation was diminished upon replacement of 4-CP with 3-CP (Figure 6-30 and Table 6-20). The *meta*-chlorophenol (3-CP) produced a 3-hour lag phase, and took 9 hours to degrade, vs. no lag phase and 8 hours for complete degradation with 4-CP. Since the biomass used in the 3-CP and 4-CP experiments was approximately the same, we concluded that the structure of the molecule must be an important contributor to the rate of halo-degradation. This could the result of evolution as the microorganisms were tailored by nature to be more suitable to attack 4-CP rather than its isomeric

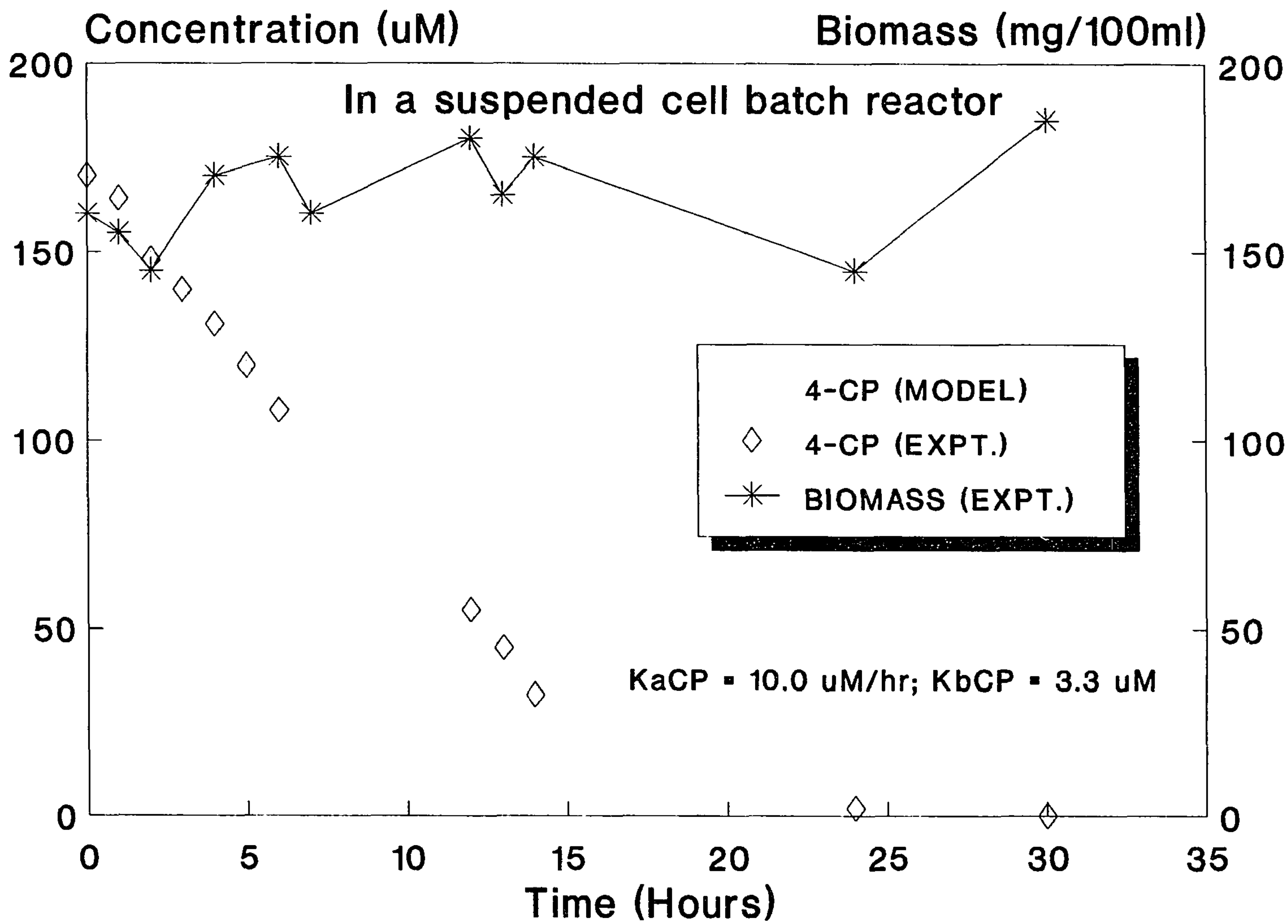

Figure 6-28 Experimental data and partially fitted curves for the aerobic mineralization of 4-CP

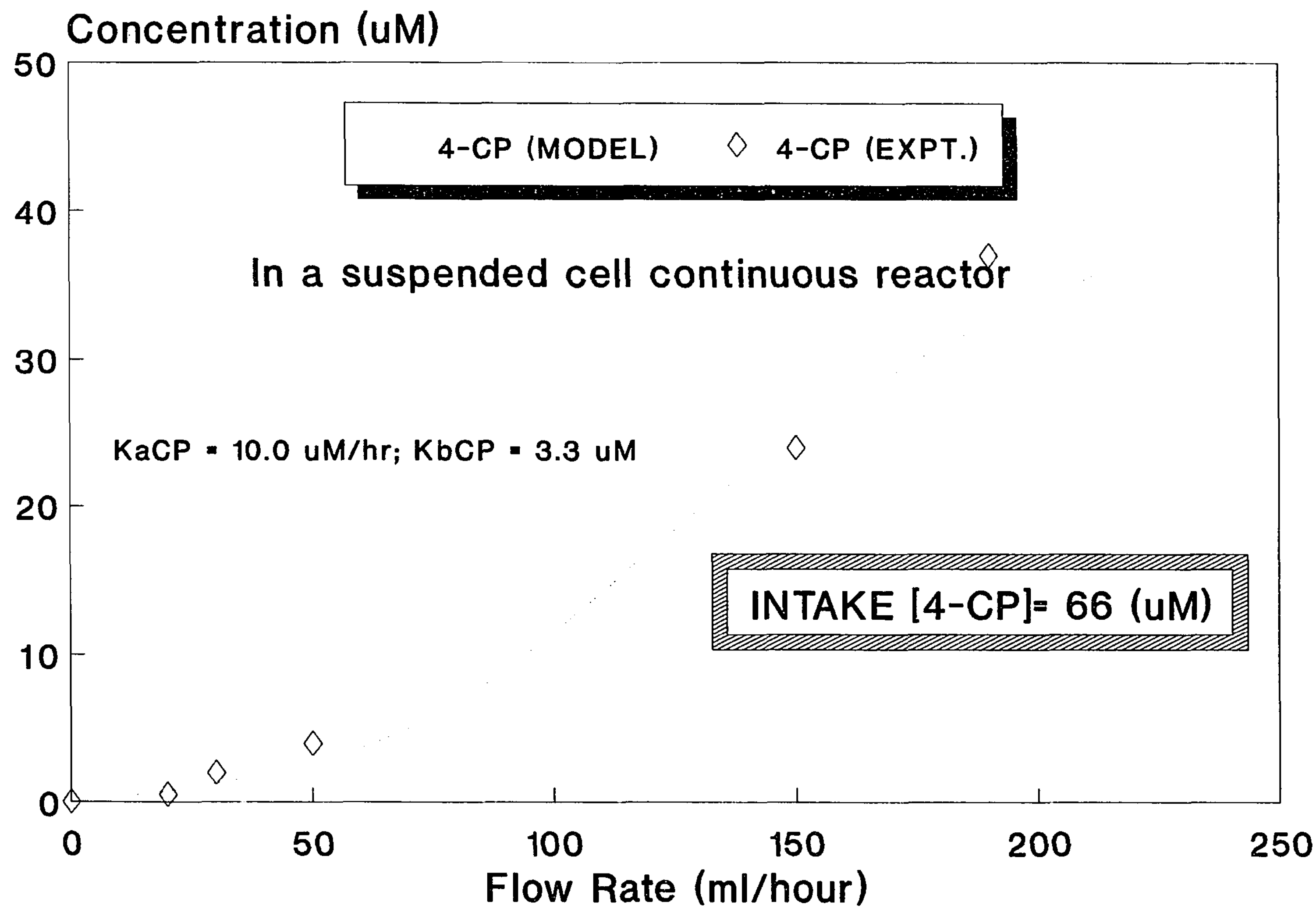

Figure 6-29 Experimental data and predicted curves for the aerobic mineralization of 4-CP

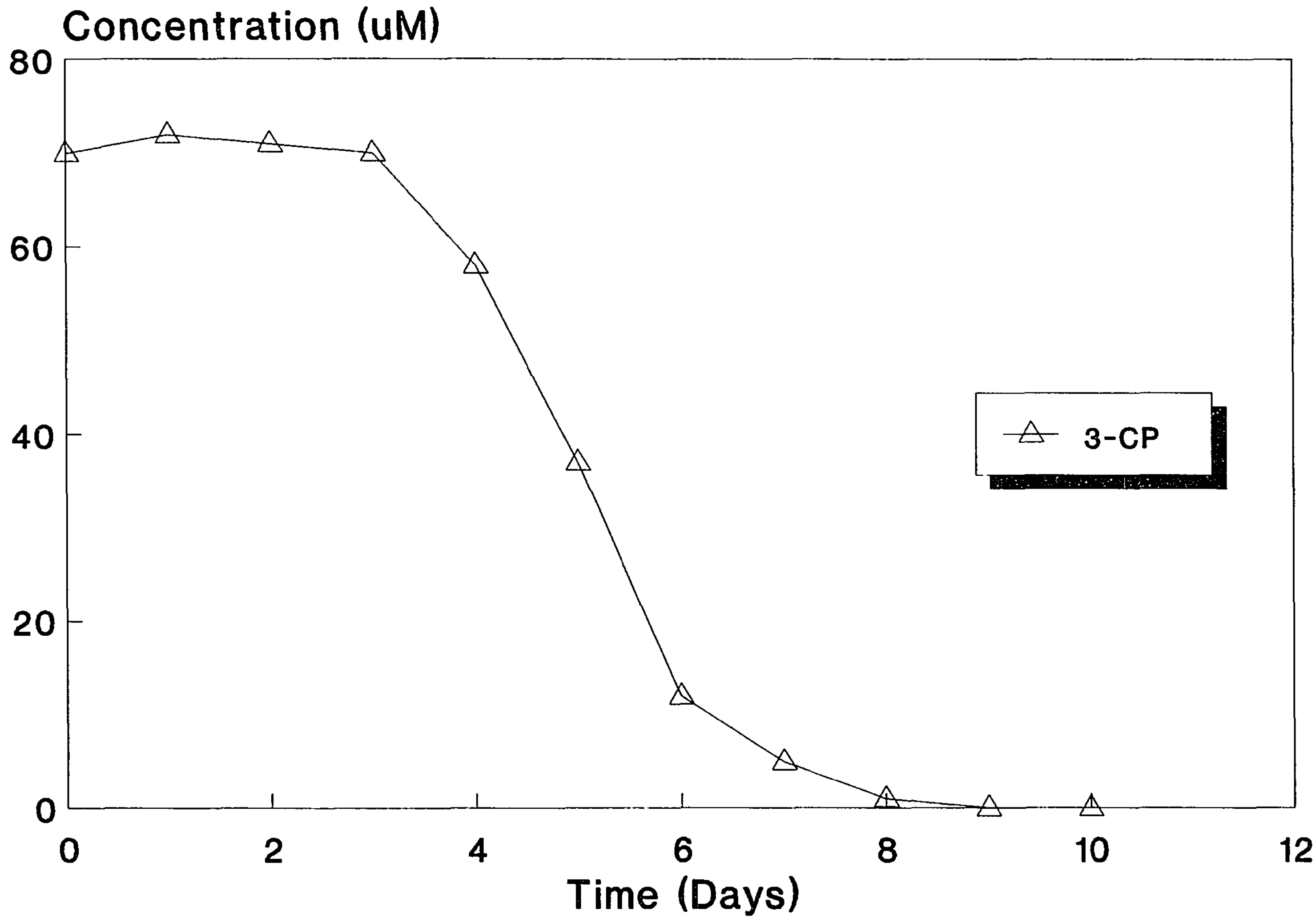

Figure 6-30 Aerobic mineralization of 3-CP in a shaker flask

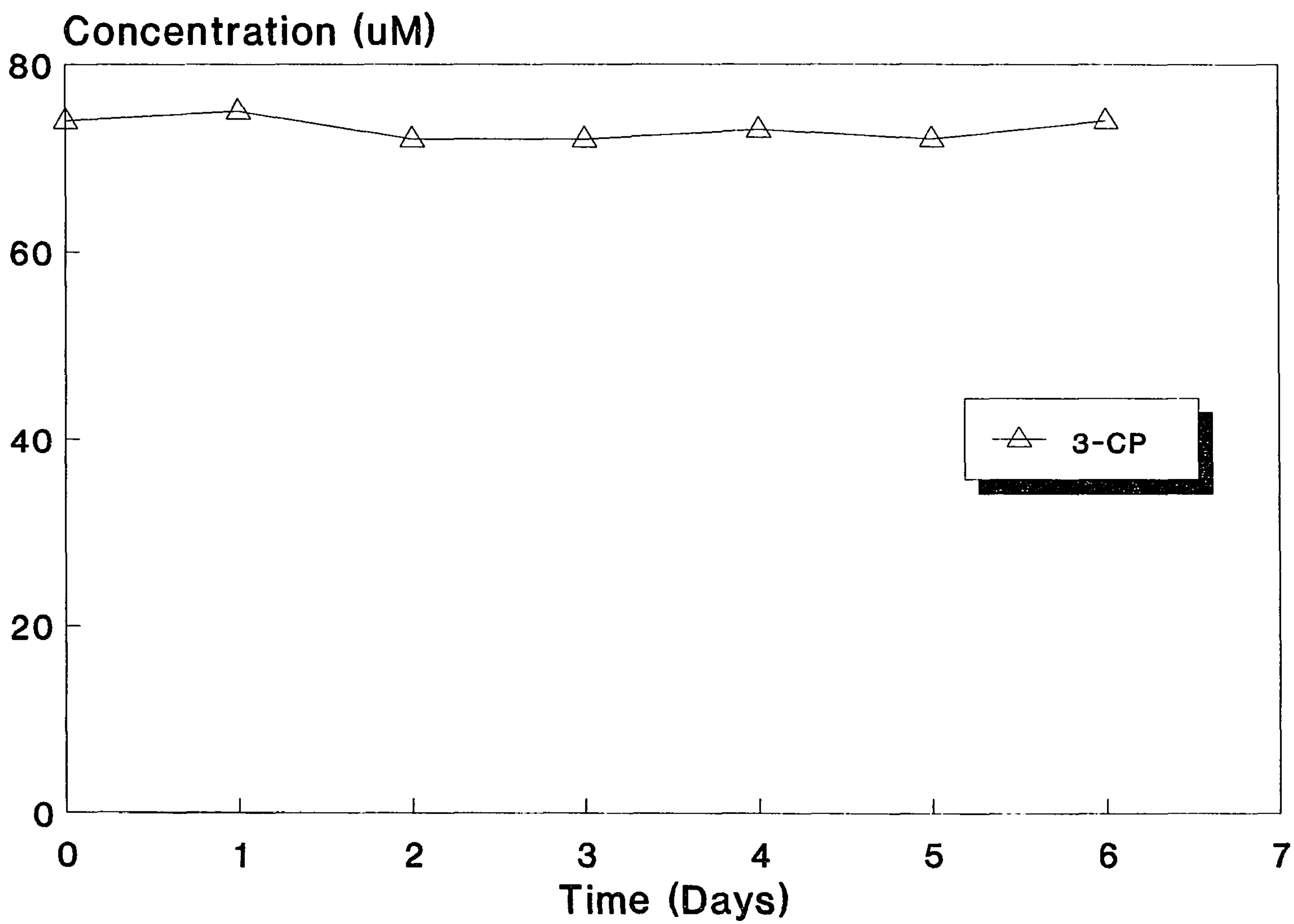

Figure 6-31 Control experiment for the aerobic mineralization of 3-CP in a shaker flask

Table 6-18 Concentration of 4-CP during the mineralization of 4-CP in an aerobic suspended cell batch reactor

Time (hours)	pH	4-CP (μM) Expt.	sum of CPs (μM)	Biomass (mg/100ml) Expt.
0	7.1	170	170	160
1	/	164	164	155
2	/	148	148	145
3	/	140	140	/
4	/	131	131	170
5	/	120	120	/
6	/	108	108	175
12	/	59	59	180
13	/	45	45	165
14	/	33	33	175
24	/	2	2	145
30	7.3	0	0	185

*** Reactor:**

Reactor volume: 770 ml
Air flow rate of reactor: 50 (ml/min)

Table 6-19 Concentration of 4-CP during the mineralization of 4-CP using an aerobic suspended cell continuous reactor, at an intake concentration of 4-CP = 66 μM

Flow Rate (ml/hr)	pH	4-CP (μM) Expt. Output	Sum of CPs (μM) Expt.
0	7.2	0	0
20	7.3	1	1
30	7.5	2	2
50	7.4	4	4
150	7.2	24	24
190	/	38	38

*** Aerobic reactor:**

Reactor volume: 770 ml

The feed to the reactor was the outlet from the anaerobic reactor. This stream contained 66 μM of 4-CP and no 2,4,6-TCP or 2,4-DCP.

Table 6-20 Concentration of 3-CP during the aerobic mineralization of 3-CP in a shaker flask

Time (Days)	3-CP (µM)	sum of CPs (µM)	CP %
0	70	70	100
1	72	72	103
2	71	71	101
3	70	70	100
4	58	58	83
5	37	37	53
6	12	12	17
7	5	5	7
8	1	1	1
9	0	0	0
10	0	0	0

*** A flask of a volume 250 ml was used as the system.**
The temperature was 30 ^{O}C

form, 3-CP. Figure 6-31 is a control experiment for abiotic losses of 3-CP. No measurable loss was observed.

6.3 Anaerobic-Aerobic Process

Figure 6-32 shows the experimental concentrations of 2,4,6-TCP, 2,4-DCP, and 4-CP at the exit of the sequential continuous anaerobic-aerobic process. The curves in this figure represent the theoretically predicted values that can be obtained integrating Equations 5.4, 5.5, and 5.14 using the values for Ka_{TCP}, Kb_{TCP}, Ka_{DCP}, and Kb_{DCP} that were obtained from batch experiments.

As the residence time decreased, less 2,4,6-TCP was dehalogenated in the anaerobic step, leading to a lower 4-CP concentration in the stream entering the aerobic reactor. A lower residence time (high flow rate) also implied that the aerobic microorganisms had less time to mineralize 4-CP, thus leading to a higher 4-CP concentration in the outlet stream, as shown in Figure 6-32.

The equations used to predict anaerobic dehalogenation and aerobic mineralization were used to predict the behavior of the entire system as a function of flow rate. Figure 6-32 and Table 6-21 presents a comparison of the experimental data and the model predictions. The results show a reasonable agreement between the experimental data and the model predictions. This agreement is even more significant if one considers that the predicted values result from a mathematical model with parameters which are derived from batch studies in which the biomass was suspended and not attached to a solid support (as in the continuous reactor system). This provides the engineer with some confidence in scaling up the anaerobic-aerobic system for treatment of halogenated aromatic compounds.

During the operation of the continuous process enough time was allowed for the system to reach steady state each time the flow rate was changed.

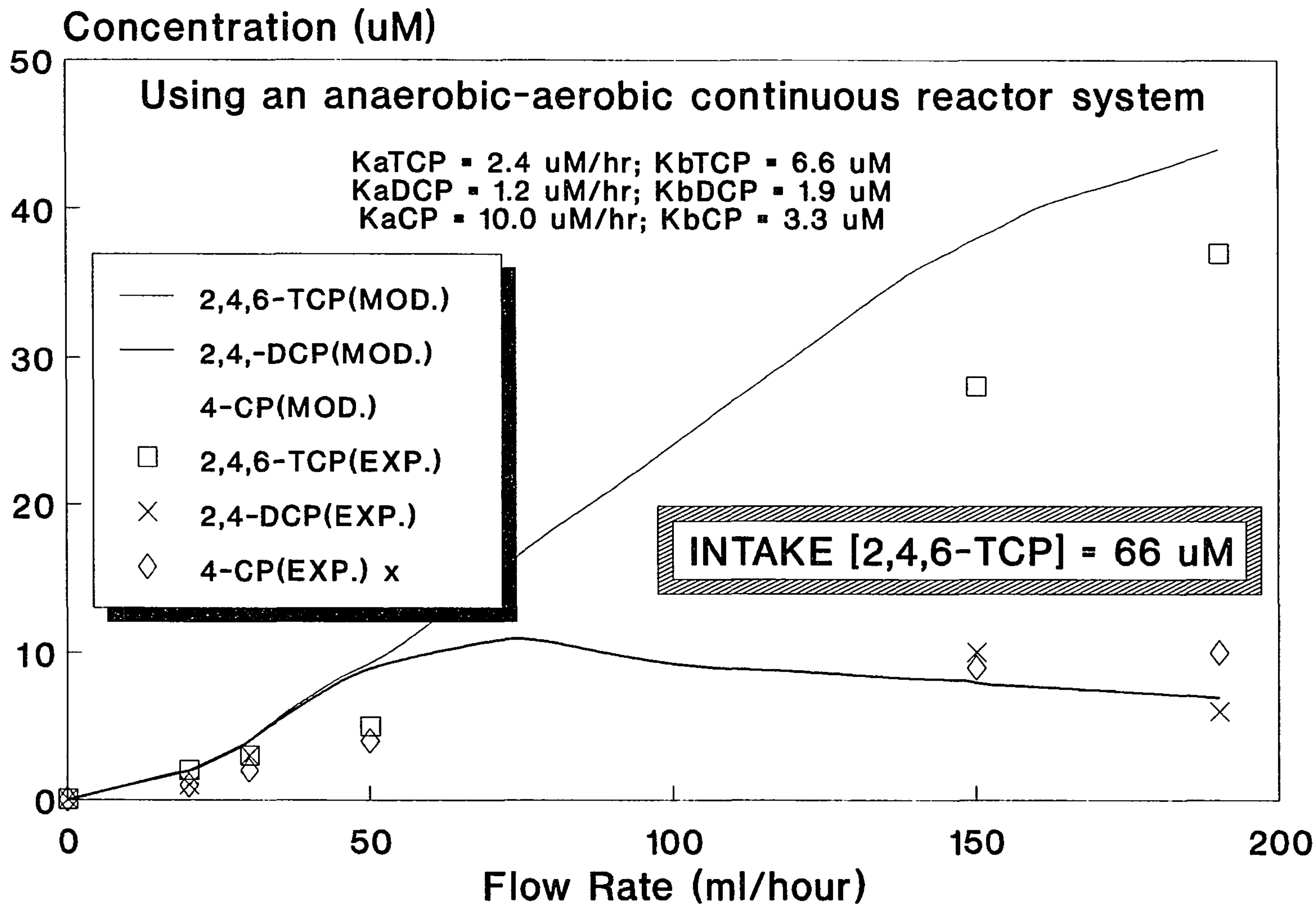

Figure 6-32 Comparsion between the predicted curves and experimental data for degradation of 2,4,6-TCP

Table 6-21 Comparison between the predicted curves and the experimental data for the anaerobic-aerobic continuous reactor system. Inlet concentration of 2,4,6-TCP = 66 μM

Flow Rate (ml/hr)	2,4,6-TCP (μM) S.S.Pred. Output	2,4,6-TCP (μM) Expt. Output	2,4-DCP (μM) S.S.Pred. Output	2,4-DCP (μM) Expt. Output	4-CP (μM) S.S.Pred. Output	4-CP (μM) Expt. Output
0	0	0	0	0	0	0
20	2	2	2	1	1	1
30	4	3	4	3	2	2
50	9	5	9	13	4	4
150	39	28	8	10	7	9
190	44	37	7	6	8	10

*** Reactor volume:**

Anaerobic reactor: 2,000 (ml) (actual liquid volume)
Aerobic reactor : 770 (ml)
Air flow rate of aerobic reactor: 50 (ml/min)

• Kinetic constants:

Anaerobic stage: K1a=2.4 (μM/hr), K2a=6.6 (μM); K1b=1.2(μM/hr), K2b=1.9 (μM)
Aerobic stage: K1= 10.0 (μM/hr), K2=3.3 (μM)

(The kinetic constants were imported from the anaerobic and aerobic batch systems.)

However, samples were taken even before the steady state had been achieved just to follow the transient process. Table 6-22 shows the concentration at the outlet of the reactor for different times after the flow rate was changed. In some cases one can clearly see that since the steady state had not yet been reached the 2,4,6-TCP concentration in the outlet was lower than that later observed when the steady state had been achieved. In order to further check that the steady state had indeed been achieved the experimental values of the outlet concentration were compared with the values that were theoretically expected using a steady state mass balance for 2,4,6-TCP in which the previously determined kinetic degradation constants had been substituted (Equation 5.4). Table 6-22 also shows a partial comparison with these predictions. The predictions are similar to the values obtained experimentally.

Table 6-22 Effect of run time of anaerobic continuous process with a constant intake concentration (68 μM) of 2,4,6-TCP, and with varied volume flow rate and initial concentration of 2,4,6-TCP.(Equation 5.4)

(Q) Volume Flow Rate (ml/hr)	(t) Run Time (hours)	[TCP] Output Exp'tal (μM)	[TCP] Output Theore. (μM)	[TCP] S. S. Theore. (μM)	$[TCP]_{in}$ Initial Conc. (μM)
30	75	2	4.15	4.16	0
45	72	6	7.9	7.9	2
50	28	15	12.4	9.46	28
80	24	9	18.8	20.6	12
80	42	25	19.9	20.6	10
99	24	18	24.6	27.0	15
99	40	29	26.9	27.0	25
150	22	28	38.3	38.7	37
190	14	37	42.4	44.0	38

Chapter 7
CONCLUSIONS

The conclusions that may be drawn from this study are:

- The halogenated aromatic compound 2,4,6-TCP can be successfully and completely mineralized by using a two-stage anaerobic-aerobic process utilizing defined media.
- The pH required in each of the two steps in the process is
  Anaerobic stage: 8.0 ~ 8.8
  Aerobic stage: 7.0 ~ 7.5.
- The degradation mechanism for both anaerobic and aerobic follows the scheme:
  2,4,6-TCP -----> 2,4-DCP -----> 4-CP
  4-CP -----> mineralization
- Stoichiometric amounts of chlorine can be recovered at the end of the two-stage process.
- The anaerobic microorganisms can be successfully immobilized on a solid porous support where they retain their dehalogenation ability.
- 2,4-DCP alone can also be degraded anaerobically. In addition, 3-CP can be degraded aerobically. No anaerobic degradation of PCP could be obtained.
- The operation of the combined reactors can be at a first approximation predicted using a mathematical model based on Michaelis-Menten kinetics.

REFERENCES

Anderson, G. K., K. J. McKeown, and T. Donnelly. 1984. "The Application of Anaerobic Packed-bed Reactors to Industrial Waste-water Treatment." *Wat. Pollut. Control.* **83**: 491-498.

Armenante, P. M., D. Kafkewitz, G. Lewandowski, and C. M. Kung. 1992. "Integrated Anaerobic-Aerobic Process for the Biodegradation of Chlorinated Aromatic Compounds." *Environ. Progr.* **11**: 113- 122.

Balch, W. E., and R. S. Wolfe. 1976. "New Approach to the Cultivation of Methanogenic Bacteria 2-Mercaptoethanesulfonic Acid (HS-CoM)-Dependent Growth of Methanobacterium Ruminantium in a Pressurized Atmosphere." *Appl. Environ. Microbiol.* **32**: 781-791.

Barnes, D. and P. A. Fitzgerald. 1987. "Anaerobic Wastewater Treatment Processes." in C. F. Forster and D. A. Wase (eds.): *Environmental Biotechnology.* Chichester, UK: Ellis Horwood. 57-113.

Battersby, N. S. and V. Wilson. 1989. "Survey of the Anaerobic Biodegradation Potential of Organic cChemicals in Digesting Sludge." *Appl. Environ. Microbiol.* **55**: 433-439.

Bedard, D .L., R. Unterman, L. H. Bopp, M. J. Brennan, M. L. Haberl, and C. Johnson. 1986. "Rapid Assay for Screening and Characterizing Microorganisms for the Ability to Degrade Polychlorinated Biphenyls." *Appl. Environ. Microbiol.* **51**: 761-768.

Beltrame, P., P. L. Beltrame, and P. Carniti. 1984. "Inhibiting Action of Chloro- and Nitro-phenols on Biodegradation of Phenol: a Structure-toxicity Relationship." *Chemosphere.* **13**: 3-9.

______1984. "Influence of Feed Concentration on the Kinetics of Biodegradation of Phenol in a Continuous Stirred Reactor." *Wat. Res.* **18**: 403-407.

Beltrame, P., P. L. Beltrame, P. Carniti, D. Guardione, and C. Lanzetta. 1987. "Inhibiting Action of Chlorophenols on Biodegradation of Phenol and its Correlation with Structuural Properties of Inhibitors." *Biotechnol. and Bioeng.* **31**: 821-828.

Berg, L. 1984. "Developments in Methanogensis from Industrial Waste Water." *Can. J. Microbiol.* **30**: 975.

Berry. D. F., A. J. Francis, and J. M. Bollag. 1987. "Microbial Metabolism of Homocyclic and Heterocyclic Aromatic Compounds under Anaerobic Conditions." *Appl. Environ. Microbiol.* **51**:43-59.

Borzelleca, J. F., L. W. Condle, and J. R. Hayes. 1984. "Toxicological Evaluation of Selected Chlorinated Phenols." in Jolley et al.(eds.): *Water Chlorination*: Chem., Environ. Impact Health Eff. Proc. Conf., 5th (Pub. 1985), Chelses, Mich. 331-343.

Boyd, S. A., D. R. Shelton. D. Berry, and J. M. Tiedje. 1983. "Anaerobic Biodegradation of Phenolic Compounds in Digested Sludge." *Appl. Environ. Microbiol.* **46**: 50-54.

Boyd, S. A., and D. R. Shelton. 1984. "Anaerobic Biodegradation of Chlorophenols in Fresh and Acclimated Sludge." *Appl. Environ. Microbiol.* **47**: 272-277.

Brown, S. C., C. P. L. Grady, and H. H. Tabak. 1990. "Biodegradation Kinetics of Substituted Phenolics: Demonstration of a Protocol Based on Electrolytic Pespirometry." *Wat. Res.* **24**: 853-861.

Bryant, M. P. 1972. "Commentary on the Hungate Technique for Culture of Anaerobic Bacteria." *Amer. J. Clin. Nutr.* **25**: 1324-1328.

Callander, I. J. and J. P. Barford. 1983. "Recent Advances in Anaerobic Digestion Technology." *Process Biochem.* **18(4)**: 24-26.

Caunt, P. and H. A. Chase. 1988. "Biodegradation by Bacteria Immobilized on Celite Particles." *Bio/Technology.* **6**: 721-725.

Chou, L. W., P. E. Speece, and R. H. Siddiqi. 1979. "Acclimation and Degradation of Petrochemical Wastewater Components by Methane Fermentation." *Biotechnol. Bioeng. Symp.* **8**: 391-394.

Chu, J. P., and E. J. Kirsch. 1972. "Metabolism of Pentachlorophenol by an Axenic Bacterial Culture." *Appl. Environ. Microbiol.* **23**: 1033-1035.

Chudoba, J., J. Albokova, B. Lentge, and R. Kummel. 1989. "Biodegradation of 2,4-Dichlorophenol by Activated Sludge Microorganisms." *Wat. Res.* **23**: 1439-1442.

Costilow, R. N., 1981. "Biophysical Factors in Growth." in Gerhardt et al.(eds.) Manual of Methods for General Bacteriology, *American Society for Microbiology,* Washington DC, 66-78.

Dalton, H., and D. I. Stirling. 1982. "Co-Metabolism." *Phi. Trans. R. Soc.* Lond. B **297**: 481-496.

DeWeerd, K. A., and J. M. Suflita. 1990. "Anaerobic Aryl Reductive Dehalogenation of Halobenzoates by Cell Extracts of *Desulfomonile tiedjei.*" *Appl. Environ. Microbiol.* **56**: 2999-3005.

Dickson, D. 1980. "Pentachlorophenol Dioxins Found to Pose Health Risks." *Nature* (London). 283-418.

Dienemann, E. A., D. S. Kosson and R. C. Ahlert. 1990. "Evaluation of Serial Anaerobic/Aerobic Packed Bed Bioreactors for Treatment of a Superfund Leachate." *J. Hazard. Mat.* **23**: 21-42.

Dietrich G. and J. Winter. 1990. "Anaerobic Degradation of Chlorophenol by an Enrichment Culture." *Appl. Microbiol. Biotechnol.* **34**: 253-258.

DiGeronimo, M. J., M. Nikaido and M. Alexander. 1979. "Utilization of Chlorobenzoates by Microbial Populations in Sewage." *Appl. Environ. Microbiol.* **37**: 619-625.

Edwards, E., E. M. Godsy, S. Henry, K. P. Mayer, and D. Grbic-Galic. 1990. *Transformation of Aromatic Hydrocarbons and Halogenated Solvents by Subsurface Microorganisms.* Presented Before the Division of Environmental Chemistry, American Chemical Society. Boston, Massachusetts. April 22-27.

Fathepure, B Z., J. M. Tiedje, and S. A. Boyd. 1988. "Reductive Dechlorination of Hexachlorobenzene to Tri- and Dichlorobenzenes in Anaerobic Sewage Sludge." *Appl. Environ. Microbiol.* **54**: 327-330.

Fathepure, B. Z., and T. M. Vogel. 1991. "Complete Degradation of Polychlorinated Hydrocarbons by a Two-stage Biofilm Reactor." *Appl. Environ. Microbiol.* **57**: 3418-3422.

Ferry, J. G. and R. S. Wolfe. 1976. "Anaerobic Degradation of Benzoate to Methane by a Microbial Consortium." *Arch. Microbiol.* **107**: 33-40.

Forday, W. and P. Greenfield. 1983. "Anaerobic Digestion." *Effl. Water Tr. J.* **23**: 405-413.

Goldman, P., G. W. A. Milne and M. T. Pignataro. 1967. "Fluorine-Containing Metabolites Formed from 2-Fluorobenzoic Acid by *Pseudomonas* Species." *Arch. Biotech. Biophys.* **118**: 178-184.

Golovleva, L. A. and G. K. Skryabin. 1981. in T. Leisinger, A. M. Cook, R. Harper, J. Nuesch (eds.): *Microbial Degradation of Xenobiotic and Recalcitrant Compounds.* Academic Press. London. 287.

Good, N. E. and S. Izawa. 1972. *Hydrogen Ion Buffers.* In: San Pietro, A. (ed) Methods in Enzymology, Volume XXIV, Photosynthesis and Nitrogen Fixation. Academic Press. New York, pp. 53-68.

Guroff, G., J. W. Daly, D. M. Jerina, J. Renson, B. Witkop, and S. Udenfriend. 1967. "Hydroxylation - Induced Migration: the NIH Shift." *Science* **157**: 1524-1530.

Hakulinen, R., S. Woods, J. Ferguson and M. Benjamin. 1985. "The Role of Facultative Anaerobic Microorganisms in Anaerobic Biodegradation of Chlorophenols." *Process Biochem.* **17**: 289-301.

Hakulinen, R., and M. Salkinija-Salonen. 1981. *An Anaerobic Fluidized-Bed Reactor for the Treatment of Industrial Wastewater Containing Chlorophenols.* in P. F. Cooper and B. Atkinson (eds.): Biological Fluidized Bed Treatment of Water and Wastewater. Chichester, UK: Ellis Horwood. p. 374.

——— 1982. "Treatment of Pulp and Paper Industry Wastewaters in an Anaerobic Fluidized Bed Reactor." *Process Biochem.* **14**: 18-22.

Hamilton, W. A. 1979. *Microbial Energetics and Metabolism.* in J. M. Lynch and N. J. Poole, (eds.): *Microbial Ecology:* A Conceptual Approach, Blackwell Scientific Publications. Oxford, U.K.

Hartmann, J., W. Reineke, H. -J. Knackmuss. 1979. "Metabolism of 3-chloro-, 4-chloro- and 3,5-dichlorobenzoate by a *Pseudomonad.*" *Appl. Environ. Microbiol.* **37**: 421-428.

Heijnen, J. J., A. Mulder, W. Enger, and F. Hoeks. 1989. "Review on the Application of Anaerobic Fluidized Bed Reactors in Wastewater Treatment." *Chem. Eng. J.* **41**: B37-B50.

Holdeman, L. V., and W. E. C. Moore. 1972. *Anaerobic Laboratory Manual.* Virginia Polytechnic Institute and State University, Blacksburg.

Holliger, C., G. Shraa, A. J. M. Stams, and A. J. B. Zehnder. 1992. "Enrichment and Properties of an Anaerobic Mixed Culture Reductively Dechlorinating 1,2,3-Trichlorobenzene to 1,3-Dichlorobenzene." *Appl. Environ. Microbiol.* **58**: 1636-1644.

Horowitz, A., J. M. Suflita, and J. M. Tiedje. 1983. "Reductive Dehalogenations of Halobenzoates by Anaerobic Lake Sediment Microorganisms." *Appl. Environ. Microbiol.* **45**: 1459-1465.

Horvath R. S. and M. Alexander. 1970. "Cometabolism of m-Chlorobenzoate by an *Arthrobacter*." *Appl. Environ. Microbiol.* **20**: 254-258.

Hungate, R. E. 1969. *A Roll Tube Method for Cultivation of Strict Anaerobes.* 117-132. In J. R. Norris and D. W. Ribbons (ed.): Methods in Microbiology Vol. 3B, Academic Press, New York.

Johnson, H. W., G. G. Briggs, and M. Alexander. 1972. "Metabolism of 3-Chlorobenzoic Acid by a *Pseudomonad*." *Soil Biol. Biochem.* **4**: 187-190.

Kafkewitz, D., P. M. Armenante, G. Lewandowski, and C. M. Kung. 1992. "Dehalogenation and Mineralization of 2,4,6-Trichlorophenol by the Sequential Activity of Anaerobic and Aerobic Microbial Populations." *Biotechnol. Letter.* **14**: 143-148.

Kennedy, K. J. and L. Berg. 1982. "Anaerobic Digestion of Piggery Waste Using a Stationary Fixed Film Reactor." *Agric. Wastes.* **4**:151.

Kiyohara, H., T. Hatta, Y. Ogawa, T. Kakuda, H. Yokoyama, and N. Takizawa. 1992. "Isolation of *Pseudomonas* Pickettii Strains that Degrade 2,4,6-Trichlorophenol and their Dechlorination of Chlorophenols." *Appl. Environ. Microbiol.* **58**: 1276-1283.

Klages, U. and F. Ligens. 1979. "Degradation of 4-Chlorobenzoic Acid by a *Nocardia* Species." *FEMS Microbiol. Lett.* **6**: 201-203.

Klecka, G. M., and J. M. Walter. 1988. "Kinetics of Microbial Growth on Mixtures of Pentachlorophenol and Chlorinated Aromatic Compounds." *Biotechnol. and Bioeng.* **31**: 328-335.

Klinman, J. P., 1972. "The Mechanism of Enzyme Catalyzed Reduced Nicotinamide Adenine Dinucleotide Dependent Reductions." *J. Biol. Chem.* **247**: 7977-7987.

Knackmuss, H. J., 1982. "Xenobiotic Degradation in Industrial Sewage: Haloaromatics as Target Substrates." *Biochem. Soc. Symp.* **48**: 173-190.

Krumme, M. L. and S. A. Boyd. 1988. "Reductive Dechlorination of Chlorinated Phenols in Anaerobic Upflow Bioreactors." *Wat. Res.* **22**: 171-177.

Kung C. M., 1991 *Integrated Anaerobic-Aerobic System for the Biodegradation of Highly Chlorinated Aromatic Compounds.* Ph. D. Dissertation, New Jersey Institute of Technology, Newark, NJ.

Larsson, P., and K. Lemkemeier. 1989. "Microbial Mineralization of Chlorinated Phenols and Biphenyls in Sediment-Water Systems from Numic and Clear-Water Lakes." *Wat. Res.* **23**: 1081-1085.

Londry, K. L., and P. M. Fedorak. 1991. "Benzonic Acid Intermediates in the Anaerobic Biodegradation of Phenols." *Can. J. Microbiol.* **38**: 1-11.

Long-de Vallere, C. L., S. Petrozzi, D. Surrer, U. Baier, and I. J. Dunn. 1989. "Methods of Anaerobic Degradation of Toxic Compounds in Chemical and Industrial Wastewaters." *Biological Waste Treatment.* 35-72.

Lovely, D. R., and M. J. Klug. 1982. "Intermediary Metabolism of Organic Matter in the Sediments of a Eutrophic Lake." *Appl. Environ. Microbiol.* **43**: 552-560.

Luong, J. H. T. 1987. "Generalization of Model Kinetics for Analysis of Growth Data with Substrate Inhibition." *Biotechnol. and Bioeng.* **29**: 242-248.

Macy, J. M., J. E. Snellen, and R. E. Hungate. 1972. "Use of Syringe Methods for Anaerobiosis." *Amer. J. Clin. Nutr.* **25**: 1318-1323.

Madsen, T. and J. Aamand. 1991. "Anaerobic Transformation and Toxicity of Tricholrophenols in a Stable Enrichment Culture." *Appl. Environ. Microbiol.* **58**: 557-561.

Madsen, T. and D. Licht. 1992. "Isolation and Characterization of an Anaerobic Chlorophenol-Transforming Bacterium." *Appl. Environ. Microbiol.* **58**: 2874-2878.

McCarty, P. L., J. S. Jeris, P. McKinney, R. E. Reed, and C. A. Wath. 1962. *Microbiology of Anaerobic Digestion.* Report R62-29, *Segwick Laboratories of Sanitary Science.* Massachusetts Institute of Technology, Camgridge, MA.

Metcalf & Eddy, Inc. 1972. *Wastewater Engineering.* McGraw-Hill New York, NY.

Mikesell, M. D., and S. A. Boyd. 1986. "Complete Reductive Dechlorination and Mineralization of Pentachlorophenol by Anaerobic Microorganisms." *Appl. Environ. Microbiol.* **52**: 861-865.

Miller, T. L., and M. J. Wolin. 1974. "A Serum Bottle Modification of the Hungate Technique for Cultivating Obligate Anaerobes." *Appl. Environ. Microbiol.* **27**: 985-987.

Mohn, W. W. and K. J. Kennedy. 1992. "Reductive Dehalogenation of Chlorophenols by *Desulfomonile tiedjei* DCB-1." *Appl. Environ. Microbiol.* **58**: 1367-1370.

______ 1992b. "Limited Degradation of Chlorophenols by Anaerobic Sludge Granules." *Appl. Environ. Microbiol.* **58**: 2131-2136.

Mohn, W. W. and J. M. Tiedje. 1990. "Strain DCB-1 Conserves Energy for Growth from Reductive Dechlorination Coupled to Formate Oxidation." *Arch. Microbiol.* **153**: 267-271.

Moos, L. P., E. J. Kirsch, R. F. Wukasch, and C. P. L. Grady. 1983. "Pentachlorophenol biodegradation-I." *Wat. Res.* **17**: 1575-1584.

Mosey, F. E. 1977. *Anaerobic Filtration: a Biological Treatment Process for Warm Trade Waste Waters: an Account of the Development of the process and Its Possible Future Applications Illustrated by Laboratory Studies of the Anaerobic Filtration of a Concentrated Carbohydrate Waste.* Tech. Rep. TR - *Water Res.* Cent (Medmenham Engl.), TR48.

Muller, R. and F. Lingens. 1986. "Microbial Degradation of Halogenated Hydrocarbons: a Biological Solution to Pollution Problems?" *Angew. Chem. Int. Ed. Engl.* **25**: 779-789.

Murthy, N. B. K., D. D. Kaufman, and G. F. Fries. 1979. "Degradation of Pentachlorophenol (PCP) in Aerobic and Anaerobic Soil." *J. Environ. Sci.* Health Part B14 1-14.

Nicholson, D. K., S. L. Woods, J. D. Istok, and D. C. Peek. 1992. "Reductive Dechlorination of Chlorophenols by a Pentachlorophenol-Acclimated Methanogenic Consortium." *Appl. Environ. Microbiol.* **58**: 2280-2286.

Neufeld, R. D., J. D. Mack, and J. P. Strakey. 1980. "Anaerobic Phenol Biokinetics." *J. Wat. Poll. Cont. Fed.* **52**: 2367-2378.

Okey R. W. and R. H. Borgan. 1965. "Apparent Involment of Electronic Mechanisms in Limiting the Microbial Metabolism of Pesticides." *J. Wat. Poll. Cont. Fed.* **37**: 692-712.

Oldenhuis, R., L. Kuijk, A. Lammers, D. B. Janssen, and B. Witholt. 1989. "Degradation of Chlorinated and Non-Chlorinated Aromatic Solvents in Soil Suspensions by Pure Bacterial Cultures." *Appl. Microbiol. Biotechnol.* **30**: 211-217.

Radehaus, Petra M. and Steven K. Schmidt. 1992. "Characterization of a Novel *Pseudomonas* sp. that Mineralizes High Concentrations of Pentachlorophenol." *Appl. Environ. Microbiol.* **58**: *2879-2885.*

Rebhun, M., and N. Galil. 1988. "Inhibition by Hazardous Compounds in an Integrated Oil Refinery." *J. Wat. Poll. Cont. Fed.* **60**: 1953-1959.

Reineke W. and H. J. Knackmuss. 1980. "Hybrid Pathway for Chlorobenzoate Metabolism in *Pseudomonas* sp. B13 Derivatives." *J. Bacteriol.* **142**: 467-473.

Rott, B., S. Nitz, and F. Korte. 1979. "Microbial Decomposition of Sodium Pentachlorophenolate." *J. Agric. Food Chem.* **27**: 306-310.

Saber, D. L. and R. L. Crawford. 1985. "Isolation and Characterization of *Flavobacterium* Strains that Degrade Pentachlorophenol." *Appl. Environ. Microbiol.* **50**: 1512-1518.

Saez, P. B., and B. E. Rittmann. 1991. "Biodegradation Kinetics of 4-Chlorophenol, an Inhibitory Co-Metabolite." *J. Wat. Poll. Cont. Fed.* **63**: 838-847.

Sahm, H., M. Brunner, and S. M. Schoberth. 1986. "Anaerobic Degradatopm of Halogenated Aromatic Compounds." *Microb. Ecol.* **12**: 147-153.

Schmidt, S. K., S. Simkins, and M. Alexander. 1985. "Models for the Kinetics of Biodegradation of Organic Compounds not Supporting Growth." *Appl. Environ. Microbiol.* **50**: 323-331.

Schonheit, P., J. Moll, and R. K. Thauer. 1979. "Nickel Cobalt and Molybdenum Requirement for Growth of *Methanobacterium Thermoautotrophicum.*" *Arch. Microbiol.* **123**: 105-107.

Shelton, D. R., and J. M. Tiedje. 1984a. "General Method for Determining Anaerobic Biodegradation Potential." *Appl. Environ. Microbiol.* **47**: 850.

——— 1984b. "Isolation and Partial Characterization of Bacteria in an Anaerobic Consortium that Mineralized 3-Chlorobenzoic Acid." *Appl. Environ. Microbiol.* **48**: 840-848.

Sleat, R. and J. P Robinson. 1984. "The Bacteriology of Anaerobic Degradation of Aromatic Compounds." *J. Appl. Bacteriol.* **57**: 381-394.

Slonim, Z., L. T. Lien, W. W. Eckenfelder, and J. A. Roth. 1985. "Anaerobic-Aerobic Treatment Process for the Removal of Priority Pollutants." *EPA Project Summary.* EPA/600/S2-85/077.

Speitel, G. E., and F. A. DiGiano. 1988. "Determination of Microbial Kinetic Coefficients through Measurement of Initial Rates by Radiochemical Techniques." *Wat. Res.* **22**: 829-835.

Speitel, G. E., C. J. Lu, M. Turakhia, and X. J. Zhu. 1989. "Biodegradation of Trace Concentrations of Substituted Phenols in Granular Activated Carbon Columns." *Environ. Sci. Technol.* **23**: 68-74.

Spokes J. R. and N. Walker. 1974. "Chlorophenol and Chlorobenzoic Acid Cometabolism by Different Genera of Soil Bacteria." *Arch. Microbiol.* **96**: 125-134.

Su, T. M. 1990. *Anoxic/oxic Two-stage Biological Treatment of Nitrite and Nitroaromatic Wastes. Presented at the Symposium of Biotechnology for Hazardous Waste Treatment.* Chinese American Academic & Professional Society (CAAPS). Annual Convention. Albany, New York.

Suflita, J. M., A. Horowitz, D. R. Shelton, and J. M. Tiedje. 1982. "Dehalogenation: A Novel Pathway for the Anaerobic Biodegradation of Haloaromatic Compounds." *Science.* **218**: 1115-1117.

Suidan, M. T., Member, ASCE, I. N. Najm, J. T. Pfeffer, Member, ASCE, and Y. T. Wang. 1988. "Anaerobic Biodegradation of Phenols Inhibition Kinetics and System Stability." *J. Environ. Eng.* **114**: 1359-1376.

Taylor, B. F., W. L. Campbell, and Chinoy. 1970. "Anaerobic Degradation of the Benzene Nucleus by a Facultatively Anaerobic Microorganisms." *J. Bacteriol.* **102(2)**: 430-437.

Tiedje, J. M., S. A. Boyd, and J. F. Quensen III. 1987. *Reductive Dechlorination of PCBs in Anaerobic Microbial Communities.* in H. L. Finkbeiner and S. B. Hamilton, (eds.): Research and Development Program for the Destruction of PCBs, *General Electric Company Corporate Research and Development.* Sixth Progress Report. Schenectady, New York. pp.29-33.

Toerien, D. F. 1967. "Enrichment Culture Studies on the Aerobic and Facultative Anaerobic Bacteria Found in Anaerobic Digester." *Wat. Res.* **1**: 147-155.

Topiwala, H. and C. G. Sinclair, 1971. "Temperature Relationship in Continuous Culture." *Biotechnol. and Bioeng.* **13**: 795-813.

Valo, R., J. Apajalathi, and M Salkinoja-Salonen. 1985. "Studies on the Physiology of Microbial Degradation of Pentachlorophenol." *Appl. Microbiol. Biotechnol.* **21**: 313-319.

Volskay, V. T., and C. P. Leslie Grady. 1990. "Respiration Inhibition Kinetic Analysis." *Wat. Res.* **24**: 863-874.

Wang, Y. T., Menber, ASCE, H. D. Gabbard, and P. C. Pai. 1991. "Inhibition of Acetate Methanogenesis by Phenols." *J. Environ. Eng.* **117**: 487-500.

Watanabe, I. 1973. "Isolation of Pentachlorophenol Decomposing Bacteria from Soil." *Soil Sci. Plant Nutr.* **19**: 109-116.

Ying, W. C., J. G. Goeddertz, W. Lin and A.S. Weber. 1990. "Biological Activated Carbon Process for Treating High Concentration Phenolic Wastewaters." Presented at the Symposium of Biotechnology for Hazardous Waste Treatment. Chinese American Academic & Professional Society (CAAPS), Annual Convention. Albany, New York.